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BRIEF REPORT

SUSHIL K. BOSE, GÖSTA ZETTERBERG and NILS FRIES: Reactivation of *Ophiostoma* cells photodynamically inactivated with visible light.

(Received November 14th, 1960)

In connection with some experiments designed to induce mutations in the fungus *Ophiostoma* by means of visible white light it appeared that the post-illumination conditions greatly influenced the number of survivors. An analysis of the results showed that the number of conidia surviving increased when the time between the end of the illumination period and the plating out of the conidia in the nutrient agar medium was increased. During this period the conidia remained in aqueous solution in the dark. Whereas effects of this sort are well-known in cells inactivated by ultraviolet light with (GEISSLER, 1959) or without (HOLLAENDER and CLAUS, 1936; ROBERT and ALDOUS, 1949) an added photoreceptor, no reactivation after treatment with visible light appears to have been observed earlier (ZELLE and HOLLAENDER, 1956; JAGGER, 1958). Therefore it seemed necessary to perform some experiments in order to establish the reality of the phenomenon.

The living material consisted of the uninucleate conidia of *Ophiostoma multiannulatum*, strain 51 ("star"), produced in continuously shaken flasks with 20 ml of liquid minimal medium N 6 (FRIES, 1949). With an inoculum of 10^8 conidia per flask the culture had just entered the stationary phase of growth after 24 hours. At that time the conidia, circa 10^{10} per flask, were centrifuged off, washed twice with distilled water, filtered through cotton-wool and resuspended in distilled water containing 0.005 per cent erythrosine. This suspension, diluted to a density of $5 \cdot 10^7$ conidia per ml was shaken mechanically in a dark room for $5\,1/2$ hours and then illuminated in a glass Büchner funnel with a sintered glass bottom through which oxygen was bubbled. The funnel was kept in a water bath at $+10°$ C. The source of illumination was a Philips, 24 volt, 150 W, lamp, placed at a distance of 7.5 cm from the glass funnel.

After 45 minutes' illumination a survival count of c. 2 per cent was obtained from platings made immediately after the illumination period. However, if the illuminated conidia were kept in the suspension for some time in darkness two to five times higher survival counts were obtained. This increase in the number of conidial survivors was studied in three experiments, the results of which are reproduced in Fig. 1.

The slower inactivation of the conidia in Expt. 3 (compared with that in the other two experiments) may have been due to inaccurate focussing of the lamp; the relatively slower increase in the number of conidial survivors in this experiment can not be explained as yet, however.

The reactivation process which occurs in the aqueous solution of erythrosine obviously comes to an end as soon as the cells are plated out in the nutrient agar medium. This could mean that the healing of the damage caused by the light requires a relatively slow metabolism. Preliminary experiments with reactivation in liquid nutrient solutions do not seem to support this interpreta-

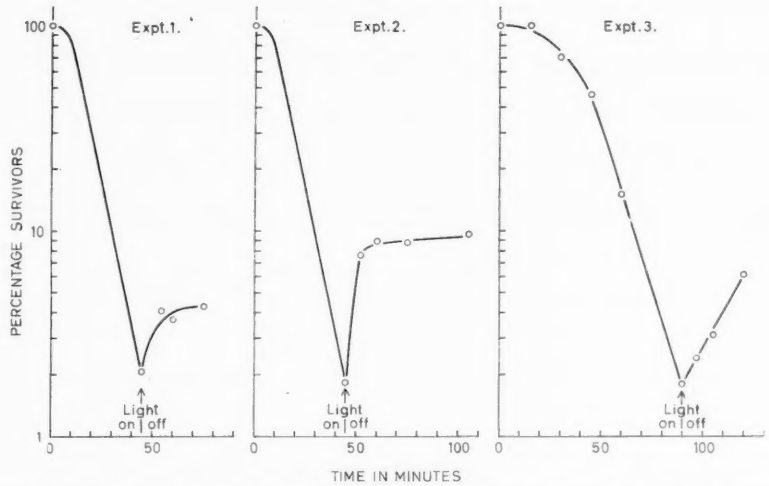

Fig. 1. The survival counts of *Ophiostoma* conidia in an aqueous solution of erythrosine after a period of illumination with visible light and during the following period in darkness.

tion. The experiments reported above do, however, show that it is also necessary to pay attention to the postillumination procedure when inactivating cells photodynamically with visible light.

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THE EFFECT OF OXYGEN CONCENTRATION ON THE FREQUENCY OF INDUCED XO MALES AND NON-DISJUNCTION FEMALES AFTER IRRADIATION OF *DROSOPHILA* MALES

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INTRODUCTION

IN recent studies by SÄVHAGEN (1960, 1961 a) concerning the relationship between the sensitivity pattern to X-ray induced damages and its relation to the treated cell stage it was found that the mating periods from the 7th—9th day after irradiation gave the highest yield of mutations (XO ♂♂ and non-disjunction ♀♀ were scored). It was further concluded that these mating periods correspond to cells which at the time of treatment were in early meiosis (prior to anaphase I). On account of these findings it is of especial interest to study the effect of the oxygen concentration upon the yield of induced genetic damages during this sensitive period.

Since BAKER and SGOURAKIS (1950) observed that the number of induced recessive lethals in *Drosophila melanogaster* males was strikingly reduced when the irradiation was performed in nitrogen atmosphere instead of air, a number of investigations have been undertaken concerning the influence of oxygen tension on different genetic damages. The major part of these studies is concentrated on mature sperm (BAKER and V. HALLE, 1953, 1955; LÜNING, 1956, 1958; LÜNING *et al.*, 1957 a, b, c; WOLFF and LINDSLEY, 1960) and the interest has been centered on the effect of oxygen concentration upon the breakage process and the reunion and/or recovery processes.

However some investigators have drawn attention to the problem of differences in sensitivity to oxygen tension in various stages of spermatogenesis. Thus OSTER, (1957, 1958 a, b) and SOBELS (1958 a, 1960),

studying the rate of induced recessive lethals and translocations, claim that the reduction of radiosensitivity after irradiation under anoxia is most pronounced in spermatids (4th—7th day a.i.). This is partly confirmed by FRITZ-NIGGLI (1958 a, b, 1959 a) who suggests that the greatest effect after treatment under anaerobic conditions is found in spermatids and spermatocytes.

On the basis of these findings and observations made by SÄVHAGEN (1961 a) the present study was undertaken in order to obtain more information about the effect of irradiation under different oxygen concentration on induced genetic damages in various stages of spermatogenesis.

MATERIAL AND METHODS

In the present investigation an attempt is made to study the effect of irradiation administered in commercial nitrogen, or oxygen, on the frequency of induced aberrations. In order to make it possible to compare earlier results obtained after irradiation in air (SÄVHAGEN, 1961 a) with the data obtained in the present study the same stocks and the same experimental techniques have been used. Thus, the females were taken from a *y w sn* ♀♀ × *y w sn*; *sc*^{8}Y ♂♂ stock, and the males from a *y*^{18} ♀♀ × *y*^{18}; *sc*^{8}Y ♂♂ stock. The occurrenc of XO males (due to induced losses of the paternal X and/or Y chromosomes) has been used as an indication of genetical damage. The induction of non-disjunction between the X and Y chromosomes in the males has been used as a criterion of early meiosis. The technique is more thoroughly discussed in a study concerning the relation between X-ray sensitivity and treated cell stages (SÄVHAGEN, 1961 a).

The males were taken out when they were 0—1 day old and were irradiated when they awoke after etherization, or they were kept without females for 3 days prior to irradiation. Immediately after irradiation the males were transferred to a mating cage. The first mating period lasted from 0 to 4 hours but from the 4th day and onwards every mating period consisted of 24 hours. Between the first mating period and the 4th day the males were kept with an excess of virgin females. The males were irradiated with a dose of 1100 r (2 min.). The irradiation was performed in a wooden box with a plexiglas top and through the box was passed a stream of commercial nitrogen or oxygen. The flies were kept 15 minutes in commercial nitrogen or oxygen before irradiation. In some experiments 0—1 day old males were irra-

diated with a dose of 4400 r (8 min.) in nitrogen atmosphere. The irradiation was administered at room temperature, otherwise stocks and experimental cultures were kept in an incubator at approximately 25° C.

RESULTS

The frequencies of induced XO males after treatment of 0—1 day old y^{1a}; sc^{8}Y ♂♂ with 1100 r in air, commercial oxygen or nitrogen and with 4400 r in commercial nitrogen are given in Table 1 (Series A and C are taken from an earlier study, SÄVHAGEN, 1961 a). Several experiments were undertaken in each series. The data are pooled in the table. The heterogeneity between the experiments is measured through chi-square tests. From Table 1 it can be seen that the frequency of observed XO males in the two control series performed in air and in commercial oxygen (Ser. A and B) do not differ statistically ($\chi^2=0.0374$; $0.90>P>>0.80$). Thus, one may conclude that oxygen treatment only does not enhance the frequency of losses of the paternal X and/or Y chromosomes. It will further be noticed that the observed rate of XO males in Series A and B is of about the same value in the different mating periods.

Turning now to the yield of induced XO males in Series D (1100 r O_2), E (1100 r N_2) and F (4400 r N_2) it is seen from Table 1 and Fig. 1 that the highest rate of XO males is obtained during the 7th—9th day after treatment, with the peak during the 8th day. This sensitivity pattern parallels Series C (1100 r air) fairly well. However, when comparing the yield of XO males after irradiation under anaerobic conditions (Series E) with the corresponding data after treatment in air (Series C), it is seen that there is a significantly lower rate of induced XO males after irradiation under anaerobic conditions. In the first mating period (0 to 4 hours a.i. — henceforth the abbreviation a.i. will be used for "after irradiation") and on the 4th day the reduction of the yield of induced XO males is about 60 %. During the following mating periods (5th—9th day after treatment), the reduction is even more pronounced (about 80—90 %) while during the 10th—11th day the reduction is about 70 %. Thus, it is evident that the reduction in oxygen tension exerts the strongest influence during the period of highest sensitivity to X-ray irradiation. This observation is consistent with the data from a study concerning the frequency of induced XO males and induced crossing-over in the third chromosome (SÄVHAGEN, 1961 b).

TABLE 1. *0—1 day old y^{16}; $sc^{8}Y$ males irradiated in air,*

Number of XO males, total number of offspring (n) and frequency of XO males in air (Ser. A) and in oxygen atmosphere (Ser. B) are also included in the table.

Series	Dose	Atm.	Time after 0—4 hrs			4th day			5th day			6th day		
			XO ♂♂	n	%	XO ♂♂	n	%	XO ♂♂	n	%	XO ♂♂	n	%
A**	—	Air	6	14,958	0.04	10	22,207	0.05	12	34,839	0.03	10	23,044	0.04
B	—	O_2	11	28,439	0.04	16	41,685	0.04	18	45,793	0.04	22	39,337	0.06
C**	1100 r	Air	94	20,538	0.46	205	62,692	0.33	377	50,552	0.75	328	24,735	1.33
D	1100 r	O_2	91	17,271	0.53*	32	7,407	0.43	296	38,089	0.78	554	64,108	0.86*
E	1100 r	N_2	4	2,164	0.18	46	33,245	0.14	72	40,801	0.18	60	24,542	0.24*
F	4400 r	N_2	6	909	0.66	103	20,466	0.50*	137	16,459	0.83*	122	16,910	0.72

The effect after irradiation with 1100 r in commercial oxygen (Series D) reveals that for mature spermatozoa (0 to 4 hours a.i.) almost no enhancing effect is observed when comparing the result with 1100 r in air (Series C). The same is also true for the 4th and 5th day after treatment. However, it must be pointed out that for the first mating period in Series D, there is a heterogeneity within the material. On the other hand, during the 6th—9th day the rate of induced XO males after irradiation in commercial oxygen (Series D) is statistically significantly lower than in Series C. On the 10th day a.i. a higher rate of XO males is observed in Series D than in Series C while on the 11th day after treatment no difference between Series C and D is observed.

During this study it was clear that the mating periods corresponding to the 7th—10th day a.i. were especially sensitive to changes in oxygen tension during irradiation and hence 8 repeats of the treatments in commercial oxygen were performed. In each experiment there is a marked increase in the rate of induced XO males from the 6th day to the 8th—9th day a.i., followed by a decrease towards the 11th day. However, through chi-square tests it is revealed that during the mating periods corresponding to the 6th—10th day a.i. there exists a heterogeneity within Series D. Thus, it is of especial interest to compare each single experiment within Series D with the pooled data and/or single experiments in Series C. Such a comparison reveals that the observed difference in the rate of induced XO males on the 6th day a.i. endures even when separate experiments in Series D and C are examined. The

oxygen or nitrogen atmosphere and mated to y w sn females.

successive mating periods are given. Data from control experiments performed in
*=heterogeneity within the material. **=data from SÄVHAGEN, 1961 a.

irradiation

7th day			8th day			9th day			10th day			11th day		
XO ♂♂	n	%	XO ♂♂	n	%	XO ♂♂	n	%	XO ♂♂	n	%	XO ♂♂	n	%
12	23,577	0.05	14	22,601	0.06	4	11,729	0.03	5	10,388	0.05	3	7,204	0.04
30	38,948	0.08	19	44,468	0.04	18	31,702	0.06	3	25,287	0.01	11	24,336	0.05
400	18,100	2.21*	463	13,350	3.47*	186	7,772	2.39*	152	27,946	0.54	137	30,044	0.46
457	32,570	1.40*	677	31,183	2.17*	427	26,021	1.64*	223	25,435	0.88*	128	25,634	0.50
158	32,715	0.48*	56	17,086	0.33*	53	14,201	0.37	38	24,900	0.15	15	11,653	0.13
154	10,662	1.44*	147	9,236	1.59	114	14,175	0.80	68	10,206	0.67	27	8,359	0.32

same is also true for the 7th—9th day a.i. where most of the obtained data in Series D are significantly lower than in Series C. On the 10th day 4 out of 8 experiments in Series D yield a significantly higher rate of observed XO males than Series C, while the remaining experiments yield results which agree with the data observed in Series C. Presumably the observed heterogeneity in Series D between the obtained data on the 10th day a.i. might depend on a biological variation between the males which results in a mixture of treated cell stages (early spermatocytes and late spermatogonial cells) becoming available for insemination during this mating period. On the basis of these results it might perhaps be more accurate to make a comparison between the total combined data for the 7th—10th day a.i. When comparing the total yield of XO males in Series C versus Series D during the above mentioned period, the following data are obtained. In Series C, 1,201 XO males out of 67,168 offspring are observed, while in Series D, 1,794 XO males are obtained out of 115,209 offspring. Through chi-square analyses it is revealed that even the total amount of induced XO males in Series D is significantly lower than in Series C. ($P<0.0005$) for the 7th—10th day a.i.

As regards the dose necessary to produce the same frequency of damages in nitrogen atmosphere as in air, SÄVHAGEN (1961 b) observed that it was necessary to increase the dose by a factor of 4. But even concerning this factor a difference in response between various mating periods was observed. Turning now to Table 1 and Fig. 1 it is seen that

from the first mating period (0 to 4 hours a.i.) until the 5th day the effect of 4400 r in nitrogen atmosphere (Series F) is slightly more effective than 1100 r in air (Series C), but from the 6th to the 9th day a.i. 1100 r in air (Series C) has a stronger effect. On the 10th—11th day after irradiation the difference between Series C and F is not statistically significant. Thus, it is evident that for the stocks used in the present experiment the effect of anoxia is most extreme in mating periods corresponding to the period of highest sensitivity.

Turning now to Table 2, Fig. 2, where the results after irradiation of 3—4 day old males are given, it is seen that the peak of induced XO males after irradiation in commercial oxygen (Series H) is reached on the 8th day after treatment, which is consistent with Series G. (1100 r in air; data from SÄVHAGEN, 1961 a.) Further comparison between Series G and H reveals that for the first mating period (mature spermatozoa) no enhancing effect is obtained after irradiation in O_2. However from the 6th day a.i. and onwards a marked increase in the yield of induced XO males is obtained in Series H as compared to Series G. Through χ^2-tests it is shown that this difference is statistically significant. These results thus indicate that for 3—4 day old males irradiation in commercial oxygen increases the yield of XO males. However, the rise in the frequency of losses of the sex-chromosomes is mainly limited to the period of highest sensitivity to X-ray irradiation. Hence, the effect of commercial oxygen on the result of irradiation of 3—4 vs 0—1 day old males is reversed as compared to the effect of air atmosphere (Table 1, Fig. 1).

When comparing Series G (1100 r air) and I (1100 r N_2) it is observed that the difference in the yield of XO males is most extreme during the period of highest sensitivity to X-ray-irradiation. This is consistent with the results for 0—1 day old males (Table 1) and in agreement with earlier findings (SÄVHAGEN, 1961 b).

An earlier study by SÄVHAGEN (1961 a) revealed that about the same sensitivity pattern for induced genetic damages is obtained after treatment of 0—1 day or 3—4 day old males. There is only a discrepancy in the amplitude of sensitivity. Turning now to Series D and H (Fig. 3) it is seen that the rate of induced XO males after irradiation in O_2 is of about the same magnitude for 0—1 and 3—4 day old males. The effect after irradiation of 3—4 day old males under anaerobic conditions (Series I) parallels also the results obtained after treatment of 0—1 day old males (Series E). The observed discrepancy on the 7th and 9th day might be due to a shift in the peak of XO males (Tables 1

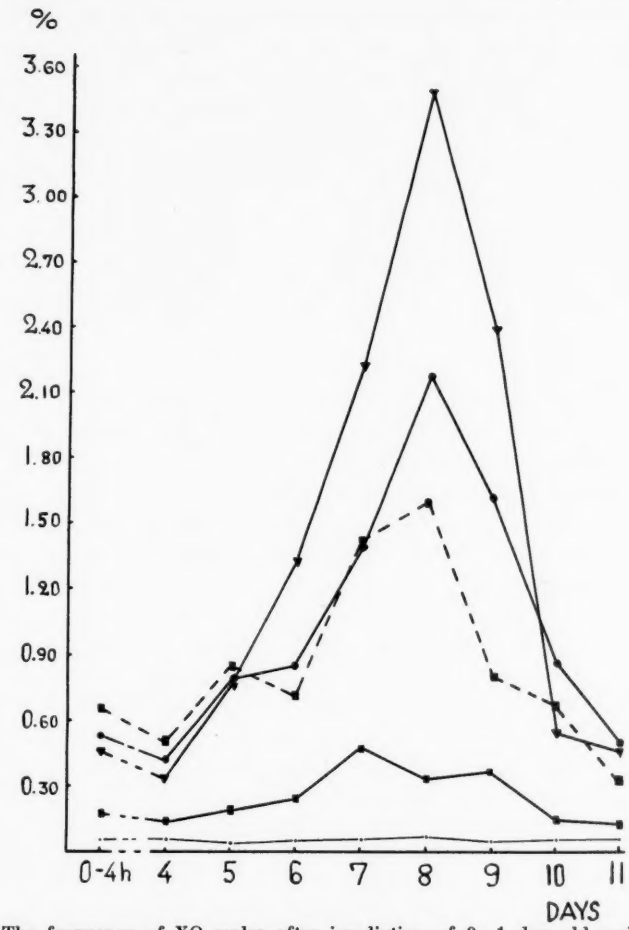

Fig. 1. The frequency of XO males after irradiation of 0—1 day old males with 1100 r in air (▼—▼); oxygen atmosphere (●—●); nitrogen atmosphere (■—■); and with 4400 r in nitrogen atmosphere (■- - -■). Control air (·—·).

and 2). Thus, the greatest difference in sensitivity to X-ray irradiation between 0—1 and 3—4 day old males seems to be obtained after treatment in air. It has to further be pointed out that contrary to results obtained after treatment of 0—1 day old males (Table 1) almost no heterogeneity is observed between the experiments after irradiation of 3—4 day old males (Table 2).

TABLE 2. *3—4 day old y^{16}; sc^8Y males given 1100 r in air,*

Number of XO males, total number of offspring (n) and percentage of XO males in
**=from SÄVHAGEN, 1961 a.

Series	Atm.	Time after											
		0—4 hrs			4th day			5th day			6th day		
		XO ♂♂	n	%	XO ♂♂	n	%	XO ♂♂	n	%	XO ♂♂	n	%
G**	Air	142	44,635	0.32	84	43,016	0.20	168	38,766	0.43	121	17,818	0.68
H	O_2	195	53,156	0.37	124	43,029	0.29	193	37,672	0.51	211	24,850	0.85
I	N_2	71	44,592	0.16	36	31,149	0.12	58	31,030	0.19	35	18,458	0.19

From SÄVHAGEN's (1961 a) study on the rate of induced non-disjunction between the paternal X and Y chromosomes it is known that the rate of wild type females rises steeply on the 7th day after irradiation, reaches a peak on the 8th day, and then decreases (Series C, Table 3). It is also evident that the rate of induced non-disjunction females is significantly lower when 3—4 day old males have been irradiated as compared to 0—1 day old males (Series C, Table 3, versus Series G, Table 4).

In Table 3 the frequency of induced non-disjunction females after irradiation of 0—1 day old males is given.

Series A and B represent two control experiments performed in air and in commercial oxygen respectively. It may be seen from Table 3 that there exists no difference between the two control experiments, hence, it is evident that treatment with commercial oxygen only, does not increase the frequency of non-disjunction. Turning now to Series D (1100 r O_2) it is seen that during the mating periods where the highest yield of non-disjunction females is obtained after irradiation in air (Series C), the rate of induced non-disjunction females after treatment in oxygen is significantly lower. However it has to be pointed out that during the 7th—10th day a.i. there exists a heterogeneity between the experiments performed in commercial oxygen. It seems therefore necessary to compare each experiment within Series D individually with the data obtained after irradiation in air. From such a comparison it is seen that in 3 of the 8 experiments performed the marked increase in the rate of induced non-disjunction females is obtained between the 6th and 7th day a.i. which is consistent with the results obtained after irradiation in air (Series C). However, in the remaining 5 experiments

oxygen or nitrogen atmosphere and mated to y w sn females.

successive mating periods are given. *=heterogeneity within the material ($P<0.05$).

irradiation

7th day			8th day			9th day			10th day			11th day		
XO ♂♂	n	%	XO ♂♂	n	%	XO ♂♂	n	%	XO ♂♂	n	%	XO ♂♂	n	%
176	14,155	1.24	181	11,706	1.55	84	8,724	0.96	41	8,312	0.49	15	7,807	0.19
165	9,723	1.70	231	9,719	2.38	190	10,736	1.77	79	10,774	0.73	119	33,191	0.36
79	26,760	0.30	89	27,466	0.32	102	21,458	0.48*	100	27,735	0.36	47	20,973	0.22

the rise is not observed until between the 7th and 8th day a.i. During the 7th and 8th day after treatment none of the experiments within Series D yields such a high percentage of induced non-disjunction females as 1100 r in air (Series C). The data obtained during the 9th day a.i. is of about the same value, irrespective of whether the irradiation was administered in commercial oxygen or in air. On the 10th day a.i. the observed rate of non-disjunction females after irradiation in oxygen is lower than in Series C. When examining the experiments individually it is seen that in 5 of 8 experiments performed in O_2 the yield of non-disjunction females has reached the control value, while in the remaining experiments the rate of non-disjunction females agree with the data obtained in Series C. Thus, it is obvious that in Series D no marked peak in the frequency of induced non-disjunction females is observed and that in most experiments the increase in the yield of non-disjunction females does not seem to occur until the 8th day after irradiation. However, if irradiation in commercial oxygen has caused a shift in the yield of induced non-disjunction females in relation to the mating periods used, it would be of especial interest to compare the total amount of non-disjunction females during the 7th—11th day after irradiation in air and in commercial oxygen. The data for Series C and D are respectively 214 non-disjunction females out of 97,212 offspring, and 179 non-disjunction females out of 140,843. Even such a comparison reveals that significantly fewer non-disjunction females are induced after treatment in oxygen ($P<0.001$).

From the present data it might be concluded that oxygen seems to increase the variability observed during the 7th—10th day a.i., but the presence of commercial oxygen during irradiation does not seem to

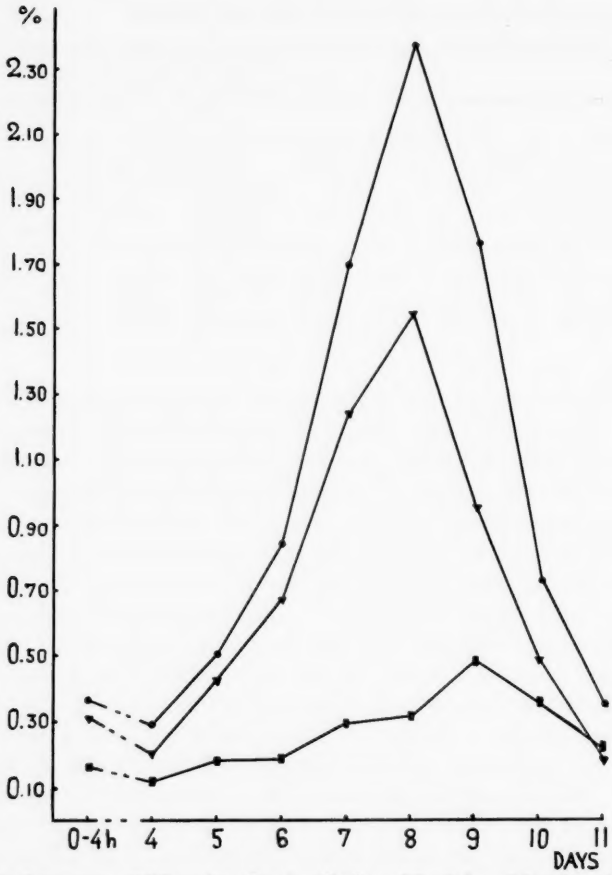

Fig. 2. The frequency of XO males after irradiation of 3—4 day old males with 1100 r in air (▼—▼); oxygen atmosphere (●—●); and nitrogen atmosphere (■—■).

increase the frequency of non-disjunction above the rate obtained after irradiation in air. Instead the results seem to point towards a "protection" against non-disjunction in the presence of an excess of oxygen during irradiation.

The rates of induced non-disjunction females after irradiation of 0—1 day old males in nitrogen atmosphere are seen in Series E and F (Table 3). From Series E (1100 r N_2) it is obvious that irradiation in commercial nitrogen reduces the rate of non-disjunction females mark-

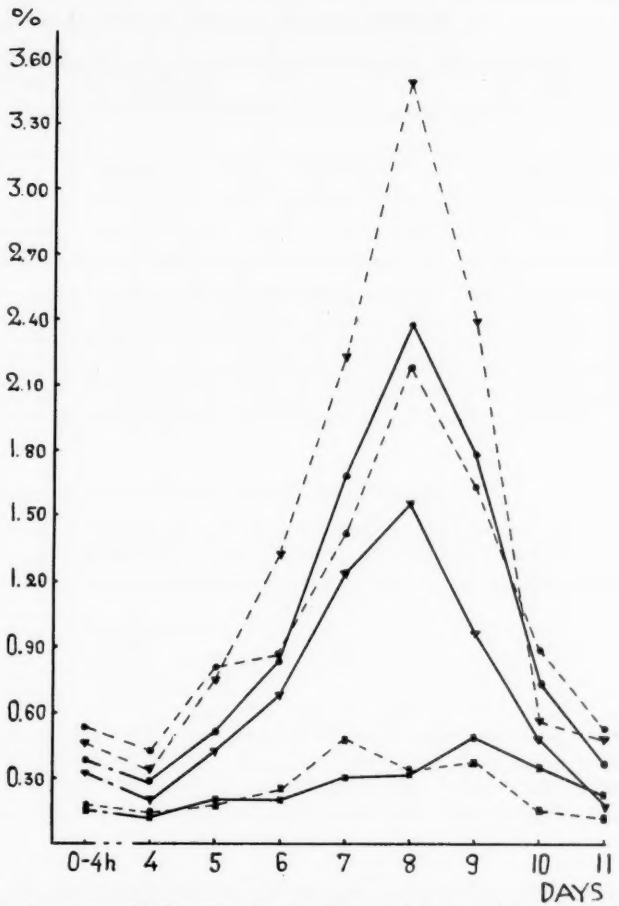

Fig. 3. The frequency of XO males after irradiation of 0—1, and 3—4, day old males with 1100 r in air, oxygen or nitrogen atmosphere. Full lines=3—4 day old males. Dashed lines=0—1 day old males. ▼—▼ or ▼---▼=air. ●—● or ●---●= oxygen, and ■—■ or ■---■=nitrogen.

edly. The difference is most drastic when a comparison is made between Series C (1100 r in air) and E, but even a comparison with Series D (1100 r O_2) gives evidence for the protective action of reduced oxygen tension during irradiation.

From Table 1 it is seen that a dose of 4400 r in nitrogen (Series F) was not able to raise the frequency of XO males to that of 1100 r in air

TABLE 3. *0—1 day old y^{1e}; $sc^{8}Y$ males given 1100 r in air, oxygen*

Number of wild type females, total number of offspring (n) and percentage of wild type females found to be due to non-disjunction is bracketed. *=heterogeneity

Series	Dose	Atm.	Time after irradiation											
			0—4 hrs			4th day			5th day			6th day		
			+ ♀♀	n	%	+ ♀♀	n	%	+ ♀♀	n	%	+ ♂♂	n	%
A**	—	Air	2 (1)	14,958	0.01	4 (3)	22,204	0.02	7 (4)	34,839	0.02	3 (1)	23,044	0.01
B	—	O_2	2 (2)	28,439	0.01	3 (1)	41,685	0.01	8 (8)	45,793	0.02	11 (11)	39,337	0.03
C**	1100 r	Air	2 (1)	20,538	0.01	20 (6)	62,692	0.03	14 (8)	50,552	0.03	2 (2)	24,735	0.01
D	1100 r	O_2	5 (4)	17,271	0.03	3 (2)	7,407	0.04	3 (2)	38,089	0.01	9 (5)	64,108	0.01
E	1100 r	N_2	2 (—)	2,164	0.05	7 (4)	33,245	0.02	12 (9)	40,801	0.03	12 (9)	24,542	0.05
F	4400 r	N_2	—	909	0.00	2 (1)	20,466	0.01	5 (1)	16,459	0.03	2 (2)	16,910	0.01

TABLE 4. *3—4 day old y^{1e}; $sc^{8}Y$ males given 1100 r in air, oxygen*

Number of wild type females, total number of offspring (n) and percentage of wild type females found to be due to non-disjunction is bracketed. **=from

Series	Atm.	Time after irradiation											
		0—4 hrs			4th day			5th day			6th day		
		+ ♀♀	n	%	+ ♀♀	n	%	+ ♀♀	n	%	+ ♀♀	n	%
G**	Air	15 (8)	44,635	0.03	16 (15)	43,016	0.04	7 (4)	38,766	0.02	6 (3)	17,818	0.03
H	O_2	9 (8)	53,156	0.02	5 (4)	43,029	0.01	2 (2)	37,672	0.01	2 (2)	24,850	0.01
I	N_2	16 (12)	44,592	0.04	3 (2)	31,149	0.01	5 (4)	31,030	0.02	5 (3)	18,458	0.03

(Series C) during the 6th—9th day a.i. When examining the rate of induced non-disjunction in Series F it is seen that the observed rate is of about the same value as 1100 r in oxygen (Series D) but lower than 1100 r in air (Series C). No pronounced peak of induced non-disjunction is observed in Series F but the higher yield of non-disjunction seems to be spread over a longer period. This might be due to the fact that irradiation in nitrogen atmosphere inhibits the cell-divisions for a period. This has been suggested by BALDWIN (1959), who claims that "the protection given by a deficiency of oxygen during exposure is associated primarily with a reduction in the numbers of cell divisions which are arrested at metaphase". However it seems as if the anaerobic condition

oxygen or nitrogen atmosphere and mated to y w sn females.

type females in successive mating periods are given. The number of progeny-tested within the material. **=from SÄVHAGEN, 1961 a.

irradiation

7th day			8th day			9th day			10th day			10th day		
+ ♀♀	n	%	+ ♀♀	n	%	+ ♀♀	n	%	+ ♀♀	n	%	+ ♀♀	n	%
6 (4)	23,577	0.03	2 (1)	22,601	0.01	1 (—)	11,729	0.01	1 (1)	10,388	0.01	1 (1)	7,204	0.01
7 (7)	38,948	0.02	13 (9)	44,468	0.03	4 (4)	31,702	0.01	6 (6)	25,287	0.02	11 (7)	24,336	0.05
40 (26)	18,100	0.22*	68 (44)	13,350	0.51	15 (9)	7,772	0.19	49 (49)	27,946	0.18	42 (29)	30,044	0.14
19 (14)	32,570	0.06*	59 (46)	31,183	0.19*	52 (41)	26,021	0.20*	27 (24)	25,435	0.11*	22 (15)	25,634	0.09
25 (13)	32,715	0.08	25 (15)	17,086	0.15	9 (6)	14,201	0.06	13 (11)	24,900	0.05	4 (2)	11,653	0.03
3 (2)	10,662	0.03	9 (7)	9,236	0.10	23 (17)	14,175	0.16	10 (8)	10,206	0.10	13 (7)	8,359	0.16

oxygen or nitrogen atmosphere and mated to y w sn females.

type females in successive mating periods are given. The number of progeny tested SÄVHAGEN, 1961 a.

irradiation

7th day			8th day			9th day			10th day			11th day		
+ ♀♀	n	%	+ ♀♀	n	%	+ ♀♀	n	%	+ ♀♀	n	%	+ ♀♀	n	%
17 (12)	14,155	0.12	25 (17)	11,706	0.21	11 (7)	8,724	0.13	12 (6)	8,312	0.14	4 (4)	7,807	0.05
11 (7)	9,723	0.11	19 (16)	9,719	0.20	25 (18)	10,736	0.23	8 (7)	10,774	0.07	28 (22)	33,191	0.08
6 (5)	26,760	0.02	10 (5)	27,466	0.04	8 (5)	21,458	0.04	23 (15)	27,735	0.08	10 (7)	20,973	0.05

exerts a protection against induced non-disjunction even at a dose of 4400 r.

In Table 4 the rates of induced non-disjunction females after treatment of 3—4 day old males under different aerobic conditions are given. From Series I (1100 r N_2) it is seen that irradiation under anaerobic conditions gives a total protection against induced non-disjunction. The effect after irradiation in commercial oxygen (Series H) parallels very well that obtained after irradiation in air (Series G). The observed difference between Series G and H during the 9th and 10th day a.i. seems to be due to a variation between the males. Thus, it is evident that irradiation in commercial oxygen does not increase the frequency

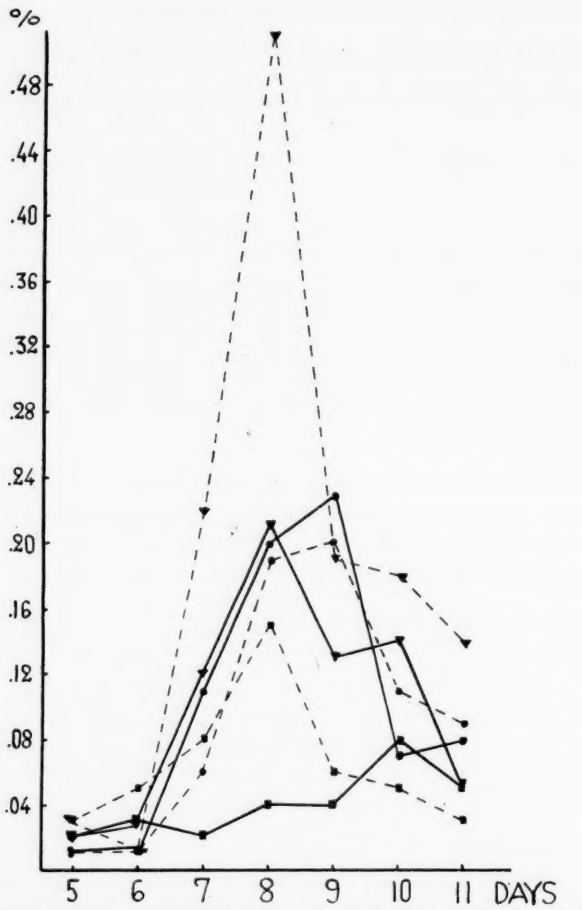

Fig. 4. The frequency of wild type females after irradiation of 0—1, and 3—4, day old males with 1100 r in air, oxygen or nitrogen atmosphere. Full lines=3—4 day old males. Dashed lines=0—1 day old males. Air=▼—▼ or ▼- - -▼; oxygen=●—● or ●- - -●; and nitrogen=■—■ or ■- - -■.

of induced non-disjunction. It has further to be pointed out that there exists no heterogeneity within the material in Table 4.

It is of especial interest to compare the rate of induced non-disjunction females after irradiation of 0—1 day old and 3—4 day old males (Fig. 4). Such a comparison reveals that after irradiation in air 0—1

day old males give a higher yield of non-disjunction females than 3—4 day old males. Irradiation administered in commercial oxygen, on the other hand, gives a rate of induced non-disjunction females of about the same magnitude for both types of males and parallels fairly well that obtained after irradiation of 3—4 day old males in air atmosphere (Ser. G). A discrepancy between 0—1 and 3—4 day old males is revealed when comparing the results after treatment in commercial nitrogen (Fig. 4). For 3—4 day old males irradiation under anoxia gives an almost total protection against induced non-disjunction, while for 0—1 day old males a reduction in the yield of non-disjunction females is observed but, still, during the 8th day a.i., a rise in wild type females is observed. Hence, the most striking difference between 3—4, and 0—1 day old males is observed after irradiation in air atmosphere, both as regards induction of non-disjunction females and of XO males.

DISCUSSION

HOLTHUSEN (1921), working with eggs of *Ascaris*, observed that organisms become more radiation resistant when the oxygen pressure was lowered. Similar experiencies were obtained on tumour cells by CRABTREE and CRAMER (1933) and, since then, the reduction of radio-sensitivity after irradiation under anoxia has been demonstrated for different materials.

GILES and BEATTY (1950), studying the percentage of induced aberrations in *Tradescantia* after irradiation under different oxygen concentrations, observed a linear increase between 0 %—10 % O_2, followed by a gradual increase to around 20 % O_2, where a plateau is reached; an observation which is confirmed by BAKER and EDINGTON (1952) and by GLASS and PLAINE (1952). GILES and RILEY (1950) have also demonstrated that the oxygen concentration had an effect only at the time of irradiation.

Turning to the present investigation, it is obvious that reduction of the oxygen tension during exposure to X-rays resulted in a decrease in the yield of damages. Further analysis concerning the reduction in the rate of XO males in the N_2-series reveals that anoxia exerts the most striking influence upon the yield of XO males during the period of highest sensitivity to X-ray irradiation (7th—9th day a.i.). An observation which is consistent with SÄVHAGEN's (1961 b) results.

Differences in "N_2-protection" between different stages in spermato- and spermiogenesis have been observed by OSTER (1957, 1958 a, b),

SOBELS (1958 a, 1960), FRITZ-NIGGLI (1958 a, b, 1959 a), and CHANG *et al.* (1959). OSTER and SOBELS, studying induced recessive lethals and translocations (OSTER, 1957, 1958 a), found that the reduction was most pronounced in spermatids (4th—7th day a.i.). FRITZ-NIGGLI, on the other hand, studying the effect of anaerobic conditions upon the yield of dominant and recessive lethals after treatment of different developmental stages of *Drosophila* males, observed the greatest difference in spermatids and spermatocytes. The latter observation is consistent with the findings of CHANG *et al.* (1959).

The discrepancy between the results in the present investigation and the above related conclusions concerning which stage in spermatogenesis is the most sensitive one to O_2-reduction during irradiation may depend on differences in experimental technique, stocks used, and type of aberration studied. The importance of these differences is discussed more thoroughly in a study concerning the relation between X-ray sensitivity and stages of development of treated cells (SÄVHAGEN, 1961 a).

In order to explain the high sensitivity of spermatids, OSTER (1957) suggested that the greater sensitivity was due to a greater availability of oxygen (intra- or intercellular) in this developmental stage. LÜNING (1954) discusses the possibility that the amplitude of the variation between various stages of spermiogenesis can be influenced by the oxygen concentration. FRITZ-NIGGLI (1959 a, b) supports the idea that the underlying cause might be a difference in intra- and/or intercellular oxygen present in these cells during irradiation. The spermatocytes and spermatids should then be more saturated with oxygen than the mature spermatozoa. Because of this, treatment with nitrogen might have a stronger influence on the spermatocytes and spermatids. FRITZ-NIGGLI further suggests that due to the low percentage of O_2 available in mature spermatozoa, any further reduction of the O_2-tension (anoxia) is hardly observable.

Turning now to the results in the present study (Tables 1 and 2) it is seen that there is a marked difference in the yield of XO males after irradiation of spermatozoa during anoxia as compared to the results after irradiation in air. It is probable that this disagreement with FRITZ-NIGGLI's results might depend on the fact that in the present experiments the irradiation effect on mature spermatozoa is only studied for the first four hours after irradiation. On the other hand, LÜNING (1954, 1956), studying the rate of induced hyperploid males and recessive lethals, observed a marked difference in the yield of genetic damage

during the first 24 hours after irradiation between experiments performed in air and those in nitrogen atmosphere.

According to the hypothesis proposed by OSTER (1957), that the greater radiosensitivity was due to a greater availability of oxygen, the effect after irradiation in almost pure oxygen atmosphere ought to be greater on mature spermatozoa. FRITZ-NIGGLI (1959 a) reports an increased rate of mutations after irradiation of mature sperm in O_2. OSTER (1958 a, b) also observed that increasing the oxygen tension above that present in air has a lesser effect on spermatids as compared with spermatozoa. SOBELS (1960) obtained the greatest effect in the first brood (1—4 days a.i.) when increasing the oxygen tension above that in air.

The results obtained in the present investigation are inconsistent with the above related data. For 0—1 day old males there is a small rise in the frequency of induced XO males after irradiation of mature spermatozoa in commercial oxygen, while for 3—4 day old males no enhancing effect is observed in the first mating period. An observation which is in agreement with ALEXANDER's (1958) findings. She reports that "oxygen concentrations over 20 % (air) were not effective for increasing the damage in sperm cells, whereas, with spermatids, atmospheres of pure oxygen continued to increase the biological damage over the values obtained for air".

The observed discrepancy in effect after irradiation in oxygen atmosphere on mature spermatozoa might depend on the above mentioned length of time for the first mating period. LÜNING (in press) studying the frequency of induced damages (XO males) in spermatozoa available for insemination at various periods during the first 24 hours a.i. observed a heterogeneity within the material (high and low yielding sperm). He was also able to demonstrate a difference between 0—1 and 3—4 day old males concerning at what time after treatment a change occurred from high to low yielding sperm. Thus, for 0—1 day old males this change already occurred within 4 hours a.i., while for 3—4 day old males the low yielding sperm did not occur until 10—12 hours a.i. In order to avoid this heterogeneity the first mating period in the present experiment was delimited to the first 4 hours a.i. However, due to this heterogeneity it seems probable that the increase of damages for irradiated spermatozoa which FRITZ-NIGGLI (1959 a) observed might be due to the fact that the low yielding sperm is more sensitive to irradiation in almost pure oxygen than the high yielding spermatozoa.

From Table 2 (irradiation of 3—4 day old males) it is furthermore

obvious that, in contrast to OSTER's (1958 a, b) findings, the greatest effect of increasing the oxygen tension above that of air is observed during the period of highest sensitivity to irradiation in air (7th—9th day a.i.). The results after irradiation of 0—1 day old males (Table 1) do neither support OSTER's and SOBELS' findings, nor parallel the results for 3—4 day old males. On the contrary, a marked reduction in the yield of XO males is obtained during the 7th—9th day a.i. in oxygen atmosphere as compared to the results obtained after treatment in air. CHANG *et al.* (1959), have also obtained a lower frequency of induced sex-linked recessive lethals in mating period D (7th—8th day a.i.) after irradiation in O_2 atmosphere as compared to irradiation in air. This observation is a parallel to ALPER's (1952) and KIMBALL's (1955) observations that oxygen can be protective.

However, it has to be pointed out that, independent of the age of the males at treatment, the rate of induced XO males after irradiation in oxygen atmosphere is of about the same value during the 6th—9th day a.i. (Fig. 3). This might be taken as an indication that one reason for the observed difference between 0—1, and 3—4, day old males seems to be the amount of oxygen available in the treated cells and/or metabolic differences. BAKER and EDINGTON (1952) have studied translocations and recessive lethals in *Drosophila* induced under various oxygen concentrations. They conclude "At low oxygen concentrations, small changes in the oxygen tension cause a rapid rise in the amount of genetic effect produced until a concentration of about 11 per cent is reached. At this point the curves break sharply and further increase in the amount of oxygen has little or no effect on the frequency with which translocations or recessive lethals are induced by a given dosage of X-radiation". On the basis of BAKER and EDINGTON's observations on *Drosophila* and GILES and RILEY's (1949) findings for *Tradescantia*, HOLLAENDER, BAKER and ANDERSSON (1951) have suggested that the sharp break between 10 and 15 per cent oxygen is probably a distinctive feature of the oxygen effect. However, the technique used by BAKER and EDINGTON (1952), with a mating period consisting of the first 6 days a.i., makes it necessary to repeat the experiment with shorter mating periods in order to avoid the bias caused by the existing heterogeneity within the sperm delivered during the first 6 days after treatment.

Because of the observed variability in response to irradiation in O_2 between different stages in spermatogenesis it seems hardly possible to compare earlier studies with the present investigation. The reason for

this is that earlier experiments differ from the present not only in the mating periods used and types of aberrations studied, but also with regard to developmental stages treated. If, as suggested above there exists a metabolic difference between 0—1, and 3—4, day old males one ought to assume that this difference in metabolic rate might be even greater between different devolopmental stages. That the over-all-metabolism of insects undergoes changes during the course of the larval-pupal-adult transformation is demonstrated for *silkworms* by SCHNEIDERMAN and WILLIAMS (1953). The existence of metabolic differences is confirmed by LUDWIG and BARSA (1957) and ROCHSTEIN (1956).

When discussing the relationship between radiosensitivity and intra- and/or intercellular oxygen available in the treated cells, OSTER (1958b) concludes that "this is probably not the only factor responsible for the differential radio-sensitivity and that other mechanisms may also be involved here since it was found that spermatozoa treated in pure oxygen are not as sensitive to the same dose of X-rays as spermatids treated in air". The idea put forward, that the observed difference in response to irradiation between 0—1, and 3—4, day old males might be due to metabolic differences, is consistent with KOROGODIN, TARUSOV and TAMBIEV's (1959) findings. They have studied the relation of recovery after irradiation to temperature and oxygen on cell suspensions of *Sacharomyces*. They suggest that the lessened metabolic activity largely inhibits developing damage. "The resting cells may recover because reactions caused by the damage are largely inhibited when the metabolic rate is low, and because toxic products produced by the radiation are destroyed. SISSAKIAN (1956), studying changes in metabolism under the effect of irradiation, observed that various links of the metabolism show different resistance to X-rays. PATT (1953) confirms the importance of the metabolic activity by the the fact that cells in mitosis are usually more susceptible to injury than the cell at rest. However, PATT points out that "there is no obvious relationship between susceptibility of different tissues and their basal oxygen consumption. Brain and kidney have higher rates of respiration than spleen, yet the former is relatively radioresistant while the latter is radiosensitive". That respiration activity may play a role in the effect of oxygen concentration during irradiation is suggested by KIHLMAN (1959). He claims that the existence of an oxygen gradient in respiring tissues explains why the X-ray sensitivity of bean roots is influenced by much lower concentrations of oxygen in the presence, than in the absence, of a respiratory

inhibitor. ZEUTHEN (1949), studying respiration in different eggs, has observed a rise in respiration beginning during the first part of mitosis with a peak around metaphase and anaphase. The existence of differences in respiration rates between different developmental stages of silkworm has been observed by ITO (1953) and for different insects by BATTELLI and STERN (1913). Through NILAN's (1954) studies on *barley* it is known that oxygen applied at low temperature reduces the mutagenic frequency.

The importance of the amount of oxygen available in treated cells and the variety of ways through which oxygen may act have been discussed by amongst others GILES (1952), READ (1952), SOBELS (1957, 1958 a, b, 1960), FRITZ-NIGGLI (1958 a, b, c, 1959 a, b), and KIHLMAN (1959). GILES (1952) supposes that the observed increase in sensitivity to irradiation in O_2 might be due to the fact that oxygen acts by favouring the production of hydrogen peroxide and that this substance may break the chromosomes. READ (1952) also calls attention to the production of toxic products *e.g.* HO_2 and H_2O_2. "The latter is known to be highly toxic, but it is also known that it can be eliminated by the catalase." FRITZ-NIGGLI (1958 b, c, 1959 a, b) confirms the idea that the damage is caused by radicals produced by irradiation. She further suggests that the mutations produced in mature spermatozoa will be caused by free OH- and H-radicals, while in immature spermatozoa the damage is due to H_2O_2 or HO_2, SOBELS (1958 b), studying post-treatment of *Drosophila* males with cyanide after X-ray irradiation, observed a significant enhancement in the rate of sex-linked lethals at high irradiation dose rate but not at low dose rate. He tentatively assumes that "H_2O_2 is produced in greater concentration at high rather than at low dose rates and that it serves as the initial step in the formation of mutagenic peroxides". However this effect is mainly restricted to stages of spermatogenesis with peak sensitivity to irradiation. SOBELS (1960) directs attention to the elimination of H_2O_2 by catalase, "Since cyanide inhibits catalase, which is responsible for the breakdown of both H_2O_2 and organic peroxides of the RO-OH type, the increase of the mutation rate in the post-treated group might be indicative of the accumulation of mutagenic peroxides produced by the irradiation". PATT (1953) has made a survey of work concerning the protective mechanism to ionizing radiation. He concludes that oxygen may convert free hydrogen atoms to the hydroperoxyl radical (HO_2). Furthermore PATT supposes that the presence of oxygen would increase the survival of free OH-radicals. As a consequence he concludes that "oxygen may be expected

to increase, and oxygen acceptors to decrease the radiosensitivity of biological systems sensitive to oxidation".

When discussing the effect of ionizing radiations on plant material usually an air/nitrogen ratio of radiosensitivity is formed (READ, 1952; EVANS, NEARY and WILLIAMSON, 1959). From results obtained in the present study one may conclude that it is not possible to talk about a common air/nitrogen ratio of radiosensitivity for irradiated *Drosophila* males, since there exists great variations between the different mating periods. This conclusion is further strengthened when studying the effect of 4400 r N_2 in different mating periods (Series F, Table 1 and Fig. 5).

The process of non-disjunction may be influenced by many factors, among them could be mentioned induced stickiness, changes in viscosity, spindle-centromere-disturbances and DNA-reproduction. In the present paper the effect of oxygen tension upon the rate of non-disjunction is studied. THODAY and READ (1949) suggest that O_2 increases the stickiness produced by X-rays. On the basis of the increased aberration frequency obtained after irradiation in oxygen, GILES (1952) discusses the idea that the effect of oxygen itself is on the recovery process. "When oxygen is present new reunions of broken ends are favored as opposed to restitution. It seemed possible, for example, that such an effect could result from the stimulation of chromosome movements by oxygen." On the basis of these suggestions one may suppose that increased chromosome movements would yield a lower percentage of non-disjunction while increased stickiness might increase the rate of non-disjunction. From the results in the present investigation it is seen that irradiation in commercial oxygen yields about the same rate of induced wild-type (non-disjunction) females independent of the age of the males at treatment (Ser. D vs Ser. H). A result which is consistent with the data obtained for XO males after treatment in oxygen. However, for 3—4 day old males the rate of induced wild type females is of about the same magnitude irrespectively of whether the irradiation was performed in air or in oxygen atmosphere. Thus, for 3—4 day old males, no enhancing or decreasing effect is observed after treatment in almost pure oxygen. On the other hand, for 0—1 day old males, irradiation in O_2 yields a lower percentage of non-disjunction females than the corresponding for males treated in air. Again, this observation parallels the results obtained for XO males after irradiation of 0—1 day old males. To draw some conclusions concerning the responsible factors for the differences between 0—1, and 3—4, day old males is

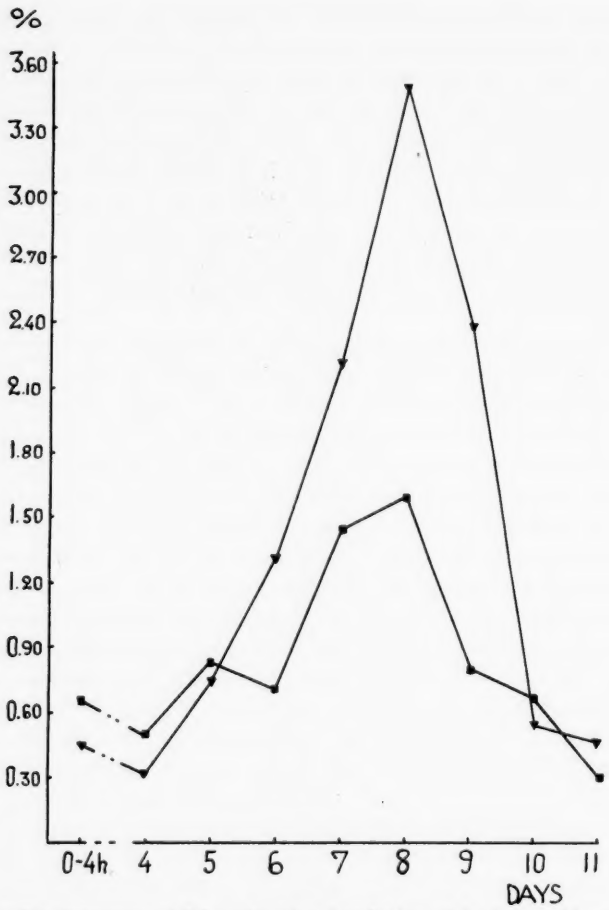

Fig. 5. The frequency of XO males after irradiation of 0—1 day old males. ▼—▼=1100 r in air; ■—■=4400 r in nitrogen.

not possible. It might, however, be permitted to call attention to GILES' (1952) idea that oxygen may stimulate chromosome movements. The above discussed results obtained after treatment of 0—1 day old males in commercial oxygen might be explained according to this hypothesis. If oxygen stimulates the chromosome movements the yield of non-disjunction females would be lower than in air. Increased chromosome movements may also increase the chances for rejoinability of induced

chromosome breaks and this in turn might result in a lower yield of induced XO males. Another possibility is that increased movements might perhaps decrease the chance for restitution and this in turn would yield a higher rate of XO males. However, whether the effect of oxygen is on the recovery process or on the breakage process is still under discussion. This problem is not dealt with in this paper.

The purpose of this study was to obtain information concerning the effect of irradiation in inducing genetic damages under different oxygen concentration during various stages of spermatogenesis.

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SUMMARY

(1) The purpose of this study is to obtain information concerning the effect of irradiation under different oxygen concentration during various stages of spermatogenesis. By use of a dual-purpose stock it was possible to study the frequency of induced XO males and induced non-disjunction between the paternal X and Y chromosomes.

(2) 0—1, and 3—4, day old y^{1e}; sc^{8}Y males were irradiated in commercial nitrogen or oxygen and mated to virgin *y w sn* females in successive mating periods. In order to make it possible to compare earlier results obtained after irradiation in air (SÄVHAGEN, 1961 a) with the data obtained in the present study the same stocks and the same experimental techniques have been used.

(3) A comparison between the yield of XO males after irradiation under anaerobic conditions and the corresponding data after treatment in air shows that a significantly lower rate of induced XO males is obtained after irradiation in N_2-atmosphere. The difference is most marked in mating periods corresponding to the period of highest sensitivity. This is consistent with SÄVHAGEN's (1961 b) observations.

(4) The sensitivity pattern to irradiation obtained after treatment in commercial oxygen parallels fairly well that obtained after irradiation in air.

(5) The effect of commercial oxygen on the frequency of induced XO males after irradiation of 0—1, and 3—4, day old males is reversed

as compared to the effect of air atmosphere. For 3—4 day old males irradiation in O_2 increases the yield of XO males. The rise in the frequency of losses of the sex-chromosomes is mainly limited to the period of highest sensitivity to X-ray irradiation. However, during the 6th—9th day a.i. the rate of induced XO males after irradiation of 0—1 day old males in O_2 is statistically significantly lower than after treatment in air.

(6) For both types of males almost no enhancing effect is obtained for mature spermatozoa after irradiation in O_2. Furthermore it is observed that the rate of induced XO males after irradiation in O_2 is of about the same magnitude for 0—1, and 3—4, day old males.

(7) As regards the dose necessary to produce the same frequency of damages in nitrogen atmosphere as in air, it is found that for the first mating periods 4400 r in nitrogen atmosphere is slightly more effective than 1100 r in air, but in mating periods corresponding to the highest sensitivity 1100 r in air has a stronger effect. From results obtained in the present study it is concluded that it is not possible to talk about a common air/nitrogen ratio of radiosensitivity for irradiated *Drosophila* males, since there exists great variations between the different mating periods.

(8) Irradiation in commercial nitrogen reduces the rate of induced non-disjunction females markedly.

(9) For 3—4 day old males, irradiation under anoxia gives an almost total protection against induced non-disjunction. For 0—1 day old males a reduction in the yield of non-disjunction females is observed, but still, on the 8th day a.i. a rise in wild type females is observed.

(10) The presence of commercial oxygen during irradiation does not seem to increase the frequency of non-disjunction above the rate obtained after irradiation in air. For 0—1 day old males the results seem to point towards a "protection" against non-disjunction in the presence of an excess of oxygen during irradiation.

(11) When comparing the rate of induced wild type females for 0—1, and 3—4, day old males after irradiation in commercial O_2, it is found that the rate of non-disjunction females is of about the same magnitude for both types of males and parallels fairly well that obtained after irradiation of 3—4 day old males in air atmosphere.

(12) The relationship between radiosensitivity and intra and/or intercellular oxygen available in the treated cells is discussed.

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THE RELATIONSHIP BETWEEN TYPE OF ABERRATION AND SENSITIVITY PATTERN IN IRRADIATED *DROSOPHILA MELANOGASTER* MALES

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IN investigations undertaken to decide which stage in spermatogenesis in *Drosophila melanogaster* is the most sensitive one to irradiation, different types of aberrations have been used as indicators of induced genetic damage. SÄVHAGEN (1961 a), has pointed out that, among other things, the aberration studied might influence the conclusion concerning the stage of highest sensitivity to irradiation. This will be further elucidated below.

A common method to study the over all genetic damage has been to use the hatchability of eggs, usually referred to as the dominant lethal-technique (LÜNING, 1952 a; STEGER, 1956; STONE, 1956; BATEMAN, 1956; FRITZ-NIGGLI, 1958; STRØMNÆS, 1959). From such studies it is evident that from the 5th day a.i. there is a marked rise in the rate of dominant lethals with a maximum between the 6th—10th day followed by a decrease on the 11th day a.i. (the abbreviation a.i. is used for "after irradiation").

Turning now to other types of aberrations studied, it is obvious that for induced sex-linked recessive lethals there is also observed an increased rate from the 4th day a.i. but the peak is reached during the 6th—7th day, followed by a sharp decrease on the 8th day a.i. (AUERBACH, 1954 a; SOBELS, 1957; BATEMAN and CHANDLEY, 1959; IVES, 1960). The same pattern is also observed for induced autosomal lethals (AUERBACH, 1954 a). Thus, the highest sensitivity to irradiation, measured through induced recessive lethals, is found in cells ready for insemination during the 6th—7th day a.i., which, according to AUERBACH

(1954 a), corresponds to cells treated in late meiosis but mainly during spermiogenesis. Another type of aberration used is autosomal translocations. For this type of aberration about the same sensitivity pattern as for sex-linked recessive lethals is obtained. Hence, the peak is reached on the 6th day a.i. followed by a sharp decrease on the 8th day a.i. (SÄVHAGEN, 1960); an observation which is consistent with the findings of AUERBACH (1954 b), CLARK (1955), SOBELS (1957), STRØMNÆS (1959), and BATEMAN and CHANDLEY (1959).

A fourth method to examine induced genetic damage has been to study viable losses of the X and Y chromosomes (the XO-technique). This method has been used for both ring- and rodshaped chromosomes by various investigators. SÄVHAGEN (1960, 1961 a, b), studying the relationship between X-ray sensitivity and stages of development of treated cells in spermatogenesis in *Drosophila*, has used the XO-technique. The author observed that from the 5th day a.i. there is an increase in the rate of induced XO males (females), the peak being reached on the 8th (9th) day, followed by a decrease towards the 11th day. These findings parallel LÜNING's (1952 c) observations for hyperploid males and BATEMAN's (1957) findings for hyperploid females. However, SÄVHAGEN (1961 a), by use of a dual-purpose stock, was able to study induced losses of the paternal sex chromosomes and induced non-disjunction between the paternal X and Y chromosomes simultaneously. In this study she observed that the rate of non-disjunction was unchanged until the 7th day a.i. where a marked rise occurred. The peak was reached on the 8th day a.i., followed by a decrease towards the 11th day. Since the occurrence of non-disjunction females was used as an indication of cells treated in early meiosis (prior to anaphase I) she concluded that sperm ready for insemination during the 7th—9th day a.i. must correspond to cells treated in early meiosis.

Induced crossing-over in the male is another type of aberration where the occurrence is limited to a certain period after irradiation. The reason for this is that, according to FRIESEN's (1936, 1937) findings, later confirmed by AUERBACH (1954 a), only single crossovers are obtained from cells treated during meiosis, while clusters of recombinants are induced in spermatogonia. On the basis of these observations, and SÄVHAGEN's (1961 a), findings that spermatozoa available for insemination during the 7th—9th day a.i. correspond to cells treated in early meiosis, it would be of interest to see during which mating period after irradiation this type of aberration will occur. SÄVHAGEN (1961 b), by use of another dual-purpose stock, studied the frequency of induced XO males

and induced autosomal crossovers in successive mating periods a.i. She observed that during the 7th—8th day a.i. only single crossovers were obtained. During the 9th—11th day mostly single recombinants are observed, but in rare cases there appeared bundles of identical or complementary crossovers. In the later mating periods clusters of crossovers are frequently observed. These findings are in agreement with AUERBACH's (1954 a), SOBELS' (1956) and IVES' (1960) observations. From these results SÄVHAGEN (1961 b) concluded that spermatozoa available for insemination from the 12th day a.i. might chiefly have been treated as spermatogonia.

From this it seems evident that the observed increase in the yield of induced crossovers on the 12th day and onwards might not be taken as a sign of greater sensitivity to irradiation. Such clustering must, instead, be taken as an indication that the recombinations have occurred in spermatogonial cells which have then undergone one or more mitotic divisions before entering meiosis.

From this review it is evident that different sensitivity patterns to X-ray irradiation could be deduced depending on which type of aberration is studied. The different sensitivity patterns are shown in Fig. 1. This figure is constructed in such a manner that all aberrations have been given the same maximal amplitude. Thus, different ordinates have been used for each type of aberration. The reason for the use of such a construction is to draw attention to the strong correlation between the types of aberrations induced and the cell stage treated, rather than to merely measure the yield of genetic damage in per cent.

From Fig. 1 it is seen that the curve representing dominant lethals circumscribes the other 4 curves, of which recessive lethals and translocations have their peaks at an earlier time than the curves for $\widehat{XX}O$ females and non-disjunction females. Hence, dominant lethals might represent several types of genetic damages and are consequently less precise than the other types of aberrations.

When comparing the obtained sensitivity patterns for induced translocations (SÄVHAGEN, 1960) and sex-linked recessive lethals (IVES, 1960) on the one hand, and for $\widehat{XX}O$ females (SÄVHAGEN, 1960) and XO males (SÄVHAGEN, 1961 a, b) on the other hand, it is evident that the observed marked decrease on the 8th day a.i. for recessive lethals and translocations may not be explained by the hypothesis proposed by AUERBACH (1954 a) that early stages of meiosis are not yet fully sensitive to the mutagenic action of X-rays. SÄVHAGEN's opinion is based on a study concerning the rate of induced XO males and non-disjunction females

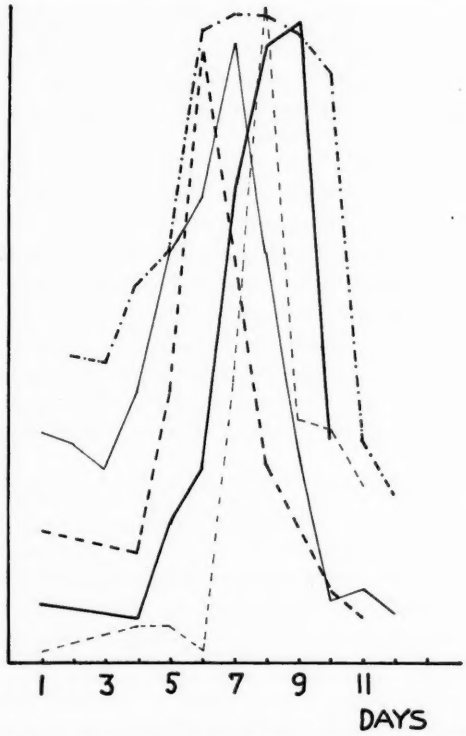

Fig. 1. Sensitivity patterns for different type of aberrations.
Dashed-dot line (—·—·—): Dominant lethals (LÜNING, 1952 a)
Heavy full line (———): XXO females (SÄVHAGEN, 1960)
Thin full line (———): Recessive lethals (IVES, 1960)
Heavy dashed line (— — —): Translocations (SÄVHAGEN, 1960)
Thin dashed line (— — —): Non-disjunction females (SÄVHAGEN, 1961 a)
(For further explanation see the text)

(1961 a), wherein she demonstrated that the observed XO males were due not only to non-disjunction but also to breakage.

Since, according to MULLER (1940), XO males are the results of elimination of the X and/or Y chromosome caused by simple breaks or reunion of the broken ends to form dicentric and acentric chromosomes, the observed decrease in the yield of translocations and recessive lethals on the 8th day a.i. might instead be due to elimination during meiosis. However, LÜNING (1952 b) has suggested that recessive

lethals might be due to intra- as well as intergenic changes, and, hence, the above drawn conclusion is only valid for recessive lethals due to intergenic changes. Thus, one may conclude that the sensitivity patterns for translocations and recessive lethals might be consistent with the pattern obtained for XO males.

If we return now to the sensitivity pattern for induced non-disjunction females (SÄVHAGEN, 1961 a) there is again an example of the strong relationship between type of aberration studied and treated cell stage. The yield of non-disjunction females after irradiation is limited to a very short period, corresponding to cells treated in early meiosis, prior to anaphase I. On the basis of SÄVHAGEN's (1961 a) observation that XO males might be the consequence of several different types of induced genetic damage, she suggested that XO males can be used as a general measure of genetic damage. From Fig. 1 it is seen that the peak in the yield of $\widehat{XX}O$ females almost coincides with the peak of non-disjunction females.

It is also obvious that even if we exclude considerations of the age of the male at treatment, the mating procedure used is of importance; a fact which has been pointed out by several investigators (LÜNING, 1952 a; AUERBACH, 1954 a; SÄVHAGEN, 1961 a). Thus, the brood-technique used seems to have a much greater importance than hitherto has been accorded it. However, it has to be pointed out that there exists also great differences in sensitivity patterns between stocks. This is demonstrated for dominant lethals and translocations by STRØMNÆS (1959).

It is also again necessary to draw attention to the importance of the age of the males at the time of treatment, since LÜNING (1952 c), and SÄVHAGEN (1961 a), have demonstrated that the amplitude of sensitivity to X-ray irradiation is dependent on the age of the male at treatment. Furthermore, LÜNING (in press) has been able to demonstrate that for mature spermatozoa also there occurs a change in sensitivity, as early as 4 hours a.i. in 0—1 day old males, while for 3—4 day old males this change does not occur until 10—12 hours a.i.

Throughout this discussion it seems evident that there exists not only a marked difference in sensitivity to irradiation between various stages of spermatogenesis in *Drosophila*, but also a difference in sensitivity according to which type of aberration is studied. Furthermore, it is obvious that the changes in sensitivity are delimited to very short intervals during spermatogenesis.

CONCLUSION

Differences between sensitivity patterns for various types of induced genetic damages have been compared and found to reflect the relationship between the different mechanisms at work in producing damages and the cell stage treated.

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QUANTITATIVE INHERITANCE AND DOMINANCE

IV. THE AVERAGE DEGREE OF DOMINANCE

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I. INTRODUCTION

METHODS of estimating the importance of dominance in the inheritance of quantitative characters have been suggested from time to time. FISHER *et al.* (1932) proposed the use of F_2's and F_3's from crosses of inbred lines. This work was further extended by MATHER (1949). COMSTOCK and ROBINSON (1948, 1952) also based their method on crosses of inbred lines. HULL's (1952) regression method presupposed crosses between a number of lines.

All these methods thus require inbred lines or at least only two alleles segregating at each locus. Their application is therefore almost exclusively restricted to naturally selfed species. So far, no method has been advanced applicable to naturally crossfertilized species.

However, in the following such a method will be given. It involves inbreeding but only to such an extent that the inbreeding is started from a random-mating population and carried on only for a few generations. The genetic quantities obtained in earlier studies on inbred populations (LAGERVALL, 1961 a and b) form the starting-point and this article merely involves a transformation of these quantities from the least-square parameters to a set of genetic parameters where the degree of dominance is included (see below). This method has its shortcomings but these shortcomings are inherent also in the methods mentioned above.

II. DEFINITION OF THE DEGREE OF DOMINANCE

Consider a locus, A, with m alleles, A_1, A_2, . . ., A_m. Let the genetic value of the homozygote A_1A_1 be α_1', the genetic value of the homozygote A_2A_2 α_2', etc., and let the numbering of the different alleles be such

that $\alpha'_1 < \alpha'_2 < \ldots < \alpha'_m$. For simplicity, consider only the two alleles A_i and A_j, where $i < j$. If the degree of dominance for the heterozygote A_iA_j is denoted by a'_{ij} the following is obtained:

Genotype:	A_iA_i	A_iA_j	A_jA_j
Genetic value:	α'_i	$\frac{\alpha'_i + \alpha'_j}{2} + a'_{ij}\frac{\alpha'_j - \alpha'_i}{2}$	α'_j

It is easily seen that if $a'_{ij}=0$ (no dominance), the heterozygote A_iA_j is intermediate to the two homozygotes. With $a'_{ij}=1$ (complete dominance) the heterozygote equals the better homozygote, A_jA_j. With overdominance, $a'_{ij}>1$, the heterozygote has a genetic value that is higher than that of the best homozygote. Partial dominance, $0<a'_{ij}<1$, indicates that the heterozygote has a value that lies between the mean of the two homozygotes and the better homozygote, A_jA_j.

Here only positive values of the degree of dominance have been considered. However, negative values can also exist. The interpretation of different negative values is obvious from the considerations above. It may be noted that the parameters used here, the genetic values of the different homozygotes and the degrees of dominance of the different heterozygotes, have been marked by primes to distinguish them from the least-square parameters used in earlier studies (LAGERVALL, 1961 a and b).

III. ESTIMATION OF THE AVERAGE DEGREE OF DOMINANCE

The discussion will for the moment be restricted to the A-locus. In the investigations on variances and covariances in inbred populations (LAGERVALL, 1961 a and b) the following five genetic quantities which were expressed in terms of the genetic least-square parameters of the original randomly mated population, were found to be of importance:

$$\sum_{i=1}^{m} p_i\,(\alpha_i)^2 = G, \qquad \sum_{i=1}^{m} p_i\,(a_{ii})^2 = D', \qquad \left(\sum_{i=1}^{m} p_i\,a_{ii}\right)^2 = D'',$$

$$\sum_{i=1}^{m}\sum_{j=1}^{m} p_i\,p_j\,(a_{ij})^2 = D, \text{ and} \qquad \sum_{i=1}^{m} p_i\,\alpha_i\,a_{ii} = C,$$

where p_i = the frequency of the gene A_i,

α_i = the effect of the gene A_i as determined by the least-square method, and

a_{ij} = the dominance deviation of the genotype A_iA_j, determined as the genetic value of $A_iA_j - \alpha_i - \alpha_j$ − the mean of the population.

It can now be shown, with rather laborious algebra, that

$$G = \frac{1}{4}R + \frac{1}{2}S - T + \frac{1}{4}U, \qquad D' = -3T + U, \qquad D'' = T,$$

$$D = T - \frac{1}{2}U + \frac{1}{2}V, \text{ and} \qquad C = -\frac{1}{2}S + 2\ T - \frac{1}{2}U,$$

where $R = \sum_{i=1}^{m-1} \sum_{\substack{j=2 \\ i<j}}^{m} p_i\, p_j\, (\alpha'_j - \alpha'_i)^2,$

$$S = \sum_{i=1}^{m-1} \sum_{\substack{j=2 \\ i<j}}^{m} p_i\, p_j\, a'_{ij} \left[(\alpha'_j)^2 - (\alpha'_i)^2\right] -$$

$$2 \sum_{i=1}^{m} p_i\, \alpha'_i \sum_{j=1}^{m-1} \sum_{\substack{k=2 \\ j<k}}^{m} p_j\, p_k\, a'_{jk}\, (\alpha'_k - \alpha'_j),$$

$$T = \left[\sum_{i=1}^{m-1} \sum_{\substack{j=2 \\ i<j}}^{m} p_i\, p_j\, a'_{ij}\, (\alpha'_j - \alpha'_i)\right]^2,$$

$$U = \sum_{i=1}^{m} \sum_{j=1}^{m} \sum_{k=1}^{m} p_i\, p_j\, p_k\, a'_{ij}\, a'_{ik} \left|\alpha'_j - \alpha'_i\right| \left|\alpha'_k - \alpha'_i\right|, \text{ and}$$

$$V = \sum_{i=1}^{m-1} \sum_{\substack{j=2 \\ i<j}}^{m} p_i\, p_j\, (a'_{ij})^2\, (\alpha'_j - \alpha'_i)^2.$$

Of these new genetic quantities only V and R are of interest in this connexion. The ratio between them gives an estimate of $\overline{(a')^2}$, the mean squared degree of dominance at the A-locus, where the squared degree of dominance of each heterozygote has been weighted according to the frequency of the heterozygote (under random mating) and to the squared difference of the genetic values of the two corresponding homozygotes. The resemblance of this ratio to the one suggested by COMSTOCK and ROBINSON (1948, 1952) for two alleles at each locus is ob-

vious; this ratio is just a generalization to any number of alleles. The square root of the ratio between V and R is then a measurement of the average degree of dominance at the A-locus.

If the different loci affecting the character under study are independent (cf. LAGERVALL, 1961 b), the quantities G, D′, D″, D, and C can be added over loci as well as the quantities R, S, etc. Division of V summed over loci by R summed over loci then yields the average squared degree of dominance for this character and the square root of this quotient thus the average degree of dominance for the character. With only two alleles present at each locus $D''=D$ (LAGERVALL, 1961 a) and thus $U=V$.

IV. DISCUSSION

The most serious shortcoming in the estimate of the average degree of dominance is that all degrees of dominance of the individual heterozygotes must be either positive or negative; if they are of both signs, the average degree of dominance will be biased. The bias is always in the direction of a larger degree of dominance than is actually the case. CHAI (1957) using MATHER's (1949) approach obtained an average degree of dominance of 1.6 for log body weight in a cross of two strains of mice, one strain selected for high and the other one for low body weight. However, other evidence from the same experiment pointed in the direction of no dominance at all or a slight partial dominance (CHAI, 1956). This can be explained by assuming that the majority of the loci affecting body weight display complete dominance, while a minority of them shows negative overdominance, *i.e.* the heterozygote is worse than either homozygote. It has further been assumed that there are only two alleles at each locus with gene frequencies 0.5 and that the effects of the different loci (as expressed in the α'-values above) are equal. Under these assumptions the genic variance and the dominance variance should be of approximately the same size, which some estimates from this experiment indicated to be the case (CHAI, 1957). Unfortunately, there is at the present time no way of testing these assumptions.

The easiest way to determine the sign of the average degree of dominance is to find out if the character shows a decrease or increase in mean at inbreeding. A decrease means a positive degree and an increase a negative degree. The reason for this is as follows. KEMPTHORNE (1957) and also LAGERVALL (1961 a) showed that, excluding epistasis, the genetic change in the mean at inbreeding is proportional to

$$\sum_{\text{loci}} \sum_{i=1}^{m} p_i \, a_{ii}$$

in the scale of least-square parameters. In the scale used here this expression =

$$-\sum_{\text{loci}} \left[\sum_{i=1}^{m-1} \sum_{\substack{j=2 \\ i<j}}^{m} p_i \, p_j \, a'_{ij} \, (\alpha'_j - \alpha'_i) \right]$$

(cf. pp. 198 and 199). Here p_i and p_j as well as $(\alpha'_j - \alpha'_i)$ are always positive. Negative values of a'_{ij} thus give an increase in the mean at inbreeding, while positive values give a decrease. Negative average degrees of dominance are therefore unlikely to occur.

Epistasis may bias the estimate of dominance to a larger or less extent. The estimation of the quantities G, D′, D″, D, and C was dealt with in an earlier publication (LAGERVALL, 1961 b). This was made under the assumption of no epistasis. It is hoped, however, that it will be possible to include genic×genic epistasis. It seems unlikely that it will be practical to include any other types of epistasis. What effect this will have upon the degree of dominance is, unfortunately, almost unknown. It seems, however, reasonable to assume with COMSTOCK and ROBINSON (1952) that epistasis probably biases the degree of dominance upwards. They estimated that with complete dominance this bias may be as large as 0.10 to 0.25.

Linkage may also have a disturbing effect. However, if G, D′, D″, D, and C are estimated from an inbreeding experiment where the original randomly mated population has been randomly bred for a number of generations (LAGERVALL, 1961 b), this effect should be negligible. It might be added that an approximate standard error of the average degree of dominance can be obtained with the help of the moment generating function of the multivariate normal distribution and with the help of the formula for the variance of a ratio.

Other things than the average degree of dominance may be estimated from the quantities R, S, etc. However, discussion of this will be postponed, until some actual data are available. An experiment with mice to investigate the importance of dominance in the inheritance of quantitative characters has been started.

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SUMMARY

The degree of dominance for a particular heterozygote is defined in the following way (any number of alleles is assumed). The genetic value of the heterozygote = the mean of the genetic values of the corresponding two homozygotes + the degree of dominance × half the difference between the genetic values of the better homozygote and the worse homozygote. No dominance corresponds to a degree of 0, partial to a degree between 0 and 1, complete dominance to a degree of 1, and overdominance to a degree larger than 1. Positive as well as negative degrees may exist.

The average degree of dominance is estimated as the square root of the average squared degree of dominance. The averaging is over all loci and over all heterozygotes possible at each locus. Each squared degree is weighted according to the frequency of the heterozygote (under random mating) and according to the squared difference between the genetic values of the corresponding two homozygotes.

Possible biases in the average degree of dominance are discussed such as the bias due to different signs of individual degrees of dominance, to epistasis and to linkage. One way of determining the sign of the average degree of dominance is given.

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TETRAD ANALYSIS AND THE COURSE OF MEIOSIS IN THREE HYBRIDS OF *LUZULA CAMPESTRIS*

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I. INTRODUCTION

THE genus *Luzula* has been proved to possess chromosomes with diffuse or non-localized centromeres. Organisms holding such chromosomes have certain cytological features in common in contrast to material possessing chromosomes with localized centromeres. Those properties characterizing chromosomes with diffuse centromeres and their effect on the course of meiosis will constitute the main subject of the present study.

The most important characteristic distinguishing organisms with diffuse centromeres from those with localized ones is the behaviour of their chromosomes during meiosis. The course of meiosis in such organisms has been described in systematically widely differing material from both animal and plant kingdoms (BATTAGLIA and BOVES, 1955). RIS (1942) has analysed the meiosis of anaphid (*Phyllaphis coweni*) and HUGHES-SCHRADER (1948) has given a review of the results concerning the *Coccids*. FEDERLEY (1943) and SUOMALINEN (1953) have studied the pairing behaviour and bivalent structure of *Lepidoptera*, and OKSALA (1943) has described the behaviour of the chromosomes for the *Odonata*. Regarding *Luzula*, several descriptions have appeared depicting the behaviour of the meiotic chromosomes in reports from DE CASTRO, CAMARA and MALHEIROS (1949), MALHEIROS, DE CASTRO and CAMARA (1947), and BROWN (1954).

In material with diffuse centromeres pairing between homologous chromosomes occurs during prophase, and chiasmata have been observed during diakinesis (BROWN, 1954; CASTRO, NORONHA-WAGNER and CAMARA, 1954; SUOMALINEN, 1953; HUGHES-SCHRADER, 1955). The chiasmata seems, however, to be completely terminalised already at first

metaphase. At this stage the most interesting observation, common to all the more thoroughly investigated materials with diffuse centromeres, is found. This is, namely, the occurrence of four distinct or "free" chromatids observed at early metaphase of the first meiotic division. At interphase the homologous chromatids associate, resulting in the paired chromatids observable during the second meiotic division. In second anaphase these paired chromatids separate. During the course of meiosis of these materials with diffuse centromeres the order of the "reductional" and the "equational" division seems to be reversed when compared with that of material with mono-centric centromeres. For the *Coccids* and other *Hemiptera* (SCHRADER, 1947) as well as for the *Odonata*, it has been possible to follow the meiotic division of the heteromorphic sex-chromosome associations and, thus, to demonstrate the fact that the first meiotic division must be morphologically equational. As regards *Luzula*, MALHEIROS, DE CASTRO and CAMARA (1947), and later on DE CASTRO (1950) assume the course of meiosis to be of the same type as for the above-mentioned insects, whereas BROWN (1954) disagrees with this assumption.

There is also another phenomenon which has been observed exclusively in material with diffuse centromeres, and by reason of which it would seem to be connected with the lack of localized centromeres. This phenomenon concerns certain characteristic modes of variation of the chromosome patterns, usually resulting in polyploid chromosome numbers. At these characteristic changes of chromosome pattern the size of the chromosomes is always reduced when their number is increased. For that reason, such changes of chromosome pattern are usually explained by some kind of chromosome fragmentation rather than by chromosome multiplication as is the case with true polyploidy. This mode of changing the chromosome patterns has been called endonuclear polyploidy in contrast to true polyploidy (NORDENSKIÖLD, 1951), MALHEIROS-GARDÉ and GARDÉ (1951) have called the phenomenon agmatoploidy, and BATTAGLIA (1955) has proposed the term pseudoploidy.

In several materials with diffuse centromeres changes of chromosome pattern have been described which must be referred to endonuclear polyploidy. This is, for instance, the case with two related species of *Thyanta* (*Hemiptera*), i.e. *T. perditor* (2n=14) and *T. calceata* (2n=28) (SCHRADER and HUGHES-SCHRADER, 1956). For these two species the contents of DNA of their spermatocyte nuclei have been tested. Approximately the same amount was found in both of them, on account of which "some type of fragmentation is assumed to be respon-

sible for the doubled chromosome number". A similar type of polyploidy has been found in several genera of *Lepidoptera* (LORKOVIC, 1941, 1949). In the genus *Polyommatus* (*Lycaena*), LORKOVIC found chromosome numbers 23, 45, and 90, and in *Lepidea* the numbers n=28, 54 and 104 were observed as well as 2n=20 and 40 in the genus *Erebia*. In these three series of closely related species the size of the chromosomes is always reduced to about half, when the chromosome number is raised to about the double. The chromosome numbers observed by LORKOVIC have been quoted in order to illustrate the fact that the endonuclear polyploid series, when complete, occurs with the basic, the doubled, and the redoubled chromosome numbers in contrast to the arithmetical series of the true polyploids. There are many other examples to which reference can be made, and SUOMALINEN (1958), in a review of this phenomenon, has reached the conclusion that it has nothing to do with true polyploidy.

As an illustration of the phenomenon of endonuclear polyploidy the variation in chromosome pattern of the *campestris-multiflora* complex of the genus *Luzula* will be used (NORDENSKIÖLD, 1949, 1951, 1956). The basic chromosome number of this complex is 6 and 2n=12 (Fig. 1 a), all the chromosomes of the genome being about the same size. This chromosome pattern is the most common one of the complex. Some species have, however, true polyploidy with 2n=24, 36, or 48 (Figs. 1 b, c and d). Here all the chromosomes are still af about the same size as that of the diploids with 2n=12. This size has been called the "standard" or AL-size of the chromosomes. Some other species of the complex may, however, also have polyploid chromosome numbers as, for instance, the doubled 2n=24 or the redoubled one, 2n=48, but here the size of the chromosomes are only a half or a quarter, respectively, of that of the standard or AL-size (Figs. 1 e and f). These small chromosomes have, in the former case, been called half-sized or BL-chromosomes (Fig. 1 e), and in the latter case quarter-sized or CL-chromosomes (Fig. 1 f). Species with these chromosome patterns are not assumed to be true polyploids in spite of their polyploid chromosome numbers. All members of the series seem to have about the same total length of chromatids and, thus, the same total nuclear contents as the diploids of the complex with 2n=12. They also easily cross with these diploids, whereas they do not cross with the true tetraploids or the true octoploids with the same chromosome numbers.

Some species of the complex have aneuploid chromosome numbers. A study of their mitotic chromosome patterns reveals, however, the

fact that some of the standard or AL-chromosomes have been replaced by two half-sized BL-chromosomes. Four such aneuploid strains of the *campestris-multiflora* complex have been found in nature. Their chromosome patterns are as follows: 2n=14 (10 AL+4 BL), 2n=16 (8 AL++8 BL) (Fig. 1 g), 2n=20 (4 AL+16 BL), and 2n=22 (2 AL+20 BL) (Fig. 1 h). These aneuploid species seem to have a half-completed endonuclear polyploidy, and just as the ones with the completed endonuclear polyploidy they belong to the diploid level of polyploidy. They may cross with each other or the 12-chromosomal diploids but never with the true polyploids of the complex. From the morphology of the mitotic chromosomes (Fig. 1) it has been assumed that two half-sized BL- or four quarter-sized CL-chromosomes of the endonuclear polyploids correspond to one standard-sized, AL-chromosome of the 12-chromosomal diploid. If this assumption is really valid the BL- or CL-chromosomes of the endonuclear polyploids must correspond to part of the large AL-chromosomes of the 12-chromosomal diploids and, consequently, these chromosomes ought to be homologous in their corresponding parts. We are then justified in presuming a pairing between those homologous parts at meiosis in hybrids between 12-chromosomal diploids and closely related endonuclear polyploids with different chromosome numbers. In order to obtain an answer to these questions such hybrids between diploids and different types of endonuclear polyploids have been produced and the chromosome pairing during the meiotic divisions has been investigated. To obtain more thorough information about the course of meiosis, the distribution of the morphologically different, large and small chromosome has been studied in the pollen tetrads in addition to the investigation of the meiotic chromosomes.

II. MATERIAL AND METHODS

Hybrids between the normal diploid *Luzula campestris, coll*, 2n=12 (12 AL) and three closely related endonuclear polyploids of different chromosome patterns have been produced and cytologically investigated. The first hybrid studied is the one between 2n=12 (AL) and 2n=16 (8 AL+8 BL) (Figs. 1 a and g). The hybrid has 2n=14 (10 AL+4 BL) (Fig. 1 i). The parents of this hybrid originate from Eastern Australia, and both taxa belong to the same species of the complex, the most common in the district, which in Australian Manuals is designed as "*L. campestris*" (EWART, 1930). It has usually 2n=12. Only three strains originating from the Brindabella district in the vicinity of Canberra, A.C.T., have been found to have 2n=16 and material of these strains has been

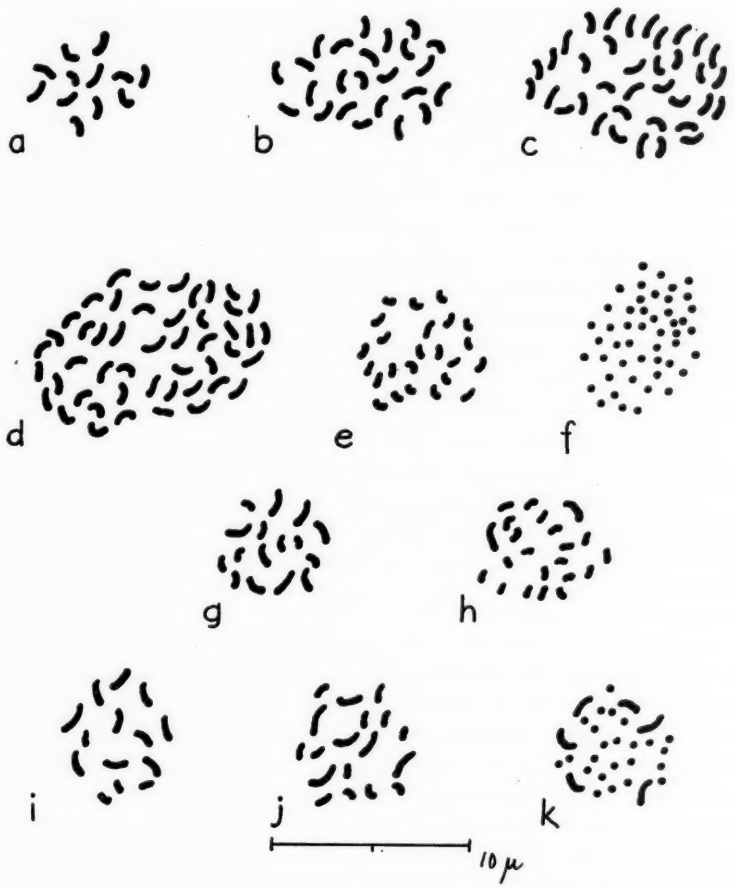


Fig. 1. Chromosome patterns occurring in the *Luzula campestris-multiflora* complex. a: the true diploid with 2n=12 AL (*L. campestris s. str.*); b: the true tetraploid with 2n=24 AL (*L. multiflora*); c: the true hexaploid with 2n=36 AL (*L. multiflora*); d: the true octoploid with 2n=48 AL (*L. congesta*); e: the endonuclear tetraploid with 2n=24 BL (the Australian "*L. australasica*"); f: the endonuclear octoploid with 2n=48 CL (*L. sudetica*); g: the aneuploid strain with 2n=16 (8 AL+8 BL) (Australian *L. campestris*); h: the aneuploid strain with 2n=22 (2 AL+20 BL) (*L. orestera*); i: the hybrid between 12 AL and (8 AL+8 BL) with 2n=10 AL+4 BL; j: the hybrid between 12 AL and 24 BL with 2n=6 AL+12 BL; k: the hybrid between 12 AL and 48 CL with 2n=6 AL+24 CL.

used in the cross as one of the parents. The seed setting ability of the hybrid is about 50 per cent.

The second hybrid studied is the one between 2n=12 (AL) and 2n=24 (BL) (Figs. 1 a and e). The hybrid has 2n=18 (6 AL+12 BL) (Fig. 1 j). One of the parents of this hybrid is the same 12-chromosomal Australian diploid as used in the first cross and the other parent is an Australian subalpine species, designated as "*L. australasica*". The strains used in the cross originate from the Snowy Mts. in New South Wales. The seed setting ability of this hybrid is about 75 per cent.

The third hybrid used for the investigation is the one lying between the two European species *L. campestris, s. str.* 2n=12 (AL) and *L. sudetica* 2n=48 (CL) (Figs. 1 a and f). The hybrid has 2n=30 (6 AL+24 CL) (Fig. 1 k). Both the parental strains used in the cross originate from Sweden. This hybrid is completely sterile.

When making these three hybrids for meiotic study the two parents of each cross have always been chosen from localities in the vicinity of each other. In this way the least possible degree of cytological disturbance will originate from the physiological and genetical differences between the parental taxa, and the characteristics of the pairing will as much as possible depend on the morphological differences of the chromosomes (NORDENSKIÖLD, 1956).

The behaviour of the chromosomes during meiosis and the first mitotic metaphase of the pollen tetrads have been studied in the three hybrids. The investigations were made with aceto-carmine smears, except for the study of the mitotic metaphase of the root tips. At first a general survey of the pairing of chromosomes at first metaphase and the behaviour of univalents at that stage was made for each hybrid. After this the study of the pollen metaphases was carried out. In this study the distributions of the large AL- and the small BL- or CL-chromosomes between the four cells of the tetrads were recorded. During this stage the four cells of the pollen tetrads still keep together in all the studied *Luzula* species met with. It is, thus, possible to investigate the chromosomal distribution from each meiosis separately. Finally, a more thorough investigation of the course of meiosis of both first and second division was performed.

III. OBSERVATIONS

1. The hybrid 2n=14 (10 AL+4 BL)

The parents of this first studied hybrid has 2n=12 AL and 2n=16 (8 AL+8 BL). The investigation of the first metaphase shows a maxi-

TABLE 1. *Pairing associations observed at first metaphase in three 14-chromosome hybrids of the (10 AL+4 BL)-constitution.*

Associations	Frequencies		
	No. 1	No. 11	No. 14
4 AL_{II}+2 AL BL_{II}	3	6	7
3 AL_{II}+2 AL+2 AL BL_{II}	4	—	2
4 AL_{II}+AL BL_{II}+AL BL+BL	19	6	14
3 AL_{II}+2 AL+AL BL_{II}+AL BL+BL	1	—	2
4 AL_{II}+2 AL BL+2 BL	4	4	5
3 AL_{II}+2 AL+2 AL BL+2 BL	1	1	—
2 AL_{II}+4 AL+2 AL BL+2 BL	1	—	—
4 AL_{II}+2 AL+4 BL	1	—	—
Total number of studied cells	34	17	30

mum pairing of six chromosome associations, indicating four homomorphic AL-bivalents and two heteromorphic associations of one long AL-chromosome and two short BL-ones. These six chromosome bodies are in early metaphase all of the same shape (Plate 2 a). In later stages of metaphase and especially in side view it is, however, possible to distinguish the two heteromorphic associations from the four homomorphic ones (Plate 2 b—g). A thorough study of the metaphase plates of three individual hybrids has given the pairing associations demonstrated in Table 1. In many cells both AL- and BL-univalents are present. But the AL-univalents originating from the homomorphic associations seem to occur in pairs and behave as if they had been paired earlier, i.e. usually occurring in the vicinity of each other (Plate 2 b). Associations of two BL-chromosomes have never been observed, and the BL-univalents very often occur in the vicinity of a large association (Plate 2 c, d). During the first metaphase the univalents are orientated in the equatorial plane and split at late metaphase into two chromatids (Plate 2 g). At anaphase each of the two chromatids migrates to its nearest pole.

To obtain a more profound knowledge of the results of meiosis of the hybrids the first mitotic metaphases of the four cells of the pollen tetrads have been studied. The chromosomes found in the four cells of a tetrad must always originate from one meiotic division. The distribution between the four cells of the large AL- and the small BL-chromosomes has been studied and recorded in Table 2. Here it is shown, in the first column, that only a low number of distribution patterns are

TABLE 2. *Distribution of large (AL) and small (BL) chromosomes in the pollen tetrads of the (10 AL+4 BL)-hybrids.*

In the table each line demonstrates a chromosomal distribution pattern. In the first column each pair of figures represents one cell, the top figures giving the number of large AL-chromosomes, the bottom figures the number of small BL-chromosomes of each of the four cells of the tetrad. (For further explanations see text.)

Chromosome distributions	No. of tetrads			Distribution of AL-chromosomes			Total no. of tetrads
	No. 2	No. 11	No. 31	No. 2	No. 11	No. 31	
AL 5—5—5—5 BL 2—2—2—2	—	12	10				
AL 5—5—5—5 BL 2—2—1—3	2	3	6	2	16	16	34
AL 5—5—5—5 BL 1—3—1—3	—	1	—				
AL 6—4—5—5 BL 0—4—2—2	7	24	15				
AL 6—4—5—5 BL 1—3—2—2	3	6	7				
AL 6—4—5—5 BL 0—4—1—3	2	3	2				
AL 6—4—5—5 BL 1—3—1—3	2	1	1	16	36	28	80
AL 6—4—5—5 BL 2—2—2—2	2	—	—				
AL 6—4—5—5* BL 0—3—3—2	—	—	1				
AL 6—4—5—5* BL 1—4—1—2	—	1	1				
AL 6—4—5—5* BL 2—3—1—2		1	1				
AL 6—4—6—4 BL 0—4—0—4	2	13	10				
AL 6—4—6—4 BL 0—4—1—3	—	3	2	6	18	12	36
AL 6—4—6—4 BL 1—3—1—3	2	2	—				
AL 6—4—6—4 BL 0—4—2—2	2	—	—				
Total number of tetrads	24	70	56				150

realized. In all, a total of 150 tetrads have been investigated, and 15 patterns of distribution have been observed. Moreover, if the distributions of only the large AL-chromosomes are taken into consideration, only three types of cells occur in the tetrads, i.e. those with 4, 5 or 6 AL-chromosomes. These figures are recorded as the four top figures of each "tetrad line" in the first column of Table 2. This very even distribution of AL-chromosomes between the four tetrad cells must depend on the regularity of chromosome pairing during meiosis. In Table 1 it is shown that four homomorphic AL-bivalents are normally formed. In the few cells where only 3 AL_{II} occur, the two AL-univalents, originating from the fourth "bivalent", must usually have been evenly distributed as well. In that way a minimum of four AL-chromosomes will always reach each tetrad cell. If the remaining two AL-chromosomes originating from the heteromorphic associations are distributed at random, i.e. in 1—1 or 2—0, the possible maximum number of AL-chromosomes in the cells will amount to 6 (4+2), and from the distribution 1—1, we will obtain the cells with 5 AL-chromosomes. If a random distribution of the two extra AL-chromosomes has really taken place, the cells with 4, 5 and 6 AL-chromosomes must occur in a ratio of 1:2:1. In the material 152:296:152 cells have been observed, and this ratio corresponds well with 1:2:1 ($0.8 > P > 0.7$).

From the study of Table 2 it is also found that the three types of cells with 4, 5 or 6 AL do not occur at random in the tetrads. That is easily understood from the fact that there exists a certain number of chromatids which have to be distributed between the cells of each tetrad. In the present hybrid the total number of AL-chromatids of the studied tetrads is always 20. With the above related conditions only three kinds of cell combinations are possible in the tetrads, i.e. 5—5—5—5, 6—4—5—5, or 6—4—6—4. All these three combination types have been observed (Table 2 and Plate 2 i—l). From the appearance of the three tetrad combinations in the material, certain conclusions concerning the course of meiosis may be drawn. As we have two meiotic divisions, one "equational" and one "reductional", we also have two different possibilities, i.e. pre- or post-reduction. (The results of possible crossing-over will be discussed later.) With the above-mentioned two postulates the distribution of the 20 AL-chromatids between the four cells of the tetrads will turn out differently. The result of the distribution of the 20 AL-chromatids with pre-reductional meiosis is demonstrated in Fig. 2 a. Here it is easily found that only two of the observed three tetrad combinations are possible to obtain after that kind of meiosis.

Fig. 2 b gives the theoretical results of post-reduction. Following that type of meiosis all three tetrad combinations in question ought to be found. From these two schedules it is easily found that post-reduction must occur if the observed conditions of the three tetrad combinations recorded in Table 2 have to be accomplished.

If the first meiotic division is morphologically equational it ought to be possible to divide the tetrads obtained into two equal halves, each of which must have a total number of 10 AL-chromosomes. These pairs of cells originating from the first meiotic division will be called "half-tetrads". Two kinds of half-tetrads are found in the present hybrid, one with 5 and 5 AL-chromosomes and the other one with 6 and 4 AL. The two kinds of half-tetrads found in the studied hybrid must be produced in an equal number, if the pairing has been regular, and, thus, the three combinations of cells in the tetrads, i.e. 5—5—5—5, 6—4—5—5, or 6—4—6—4, ought to occur in a ratio of 1:2:1. The ratio observed in the material is 34:80:36, which gives good agreement when compaired with 1:2:1 ($0.5 > P > 0.3$). In Table 2 the cells of the tetrads have been furnished in such an order that the two first ones and the two last ones taken together will always form a half-tetrad.

Concerning the distribution of the BL-chromosomes the following may be presumed. If the postulated pairing between one AL- and two BL-chromosomes during meiosis is true, then the reductional division must give a random distribution of pairs of BL-chromosomes. In this manner two BL-chromosomes, when occurring in the tetrad cells, ought to play the role of partner to each of the randomly distributed two AL-chromosomes. In this way two BL-chromosomes will replace one AL-chromosome, just as they have done in the somatic cells of the original natural strains and in the hybrids. Thus, cells with four AL-chromosomes ought to have four (2×2) additional BL-chromosomes, cells with five AL must have two (1×2) BL-chromosomes and those with 6 AL will occur without any BL-chromosomes. This substitutional system also seems to work in the majority of the studied cells. In 458 of the 600 investigated cells, i.e. 76 per cent, we really have each lacking AL-chromosome replaced by 2 BL-chromosomes. These facts demonstrate that there really must have been a fairly regular chromosome pairing in the hybrids during at least one of the meiotic divisions, and that this pairing ought to have been of the postulated type with the described four homomorphic and two heteromorphic "bivalents".

In many tetrads, i.e. 24 per cent of the investigated cells, we find, however, deviations from the described mode of distribution of the

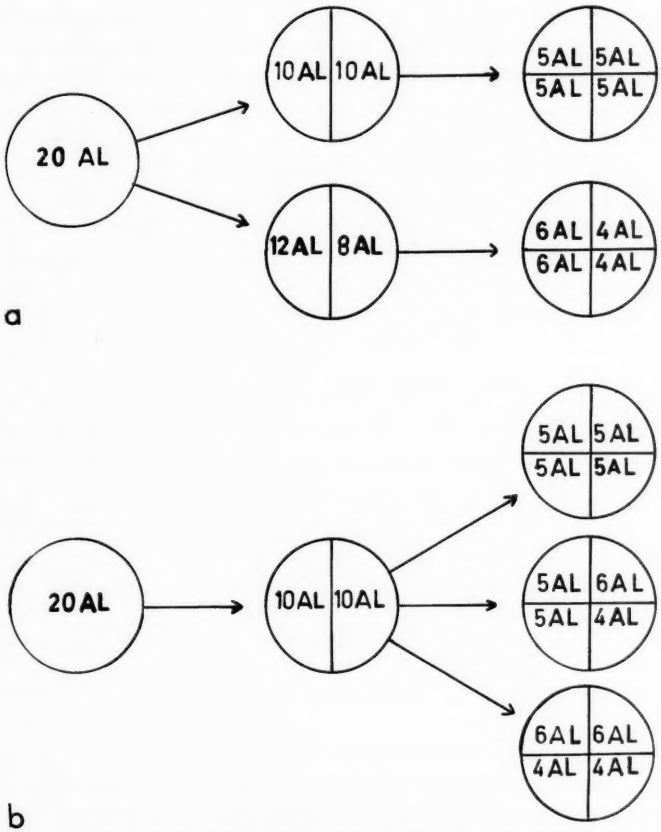


Fig. 2. Two models of tetrad formation showing the possible distributions of the 20 AL chromatids in the (10 AL+4 BL)-hybrid after a: pre-reduction; b: post-reduction.

small BL-chromosomes. Such deviations in the distribution of chromosomes between the four cells of the tetrads may arise in first or in second meiotic division. The most likely explanation for the disturbances during the first division is the occurrence of incomplete chromosome pairing. Such irregularities have been observed during the first metaphase (Table 1). The BL-univalents formed in cells with incomplete pairing split during first metaphase, each chromatid usually migrating to its nearest pole. In this way we almost always obtain an accurate equational first division, resulting in 10 AL+4 BL in each

dyad or half-tetrad. Thus, the occurrence of univalents at first metaphase usually does not change the picture of that distribution pattern. Only five of the 150 investigated tetrads have a distribution which does not show two equal half-tetrads. These tetrads are marked with an asterisk in Table 2. The distribution patterns of these tetrads is, however, easily explained by assuming that both the chromatids of a BL-univalent have migrated to one and the same pole.

The distribution of BL-chromosomes between the two cells of the half-tetrads, i.e. the distribution during the second meiotic division, can, however, not always have been regular. Sometimes one of the BL-chromatids migrates to the wrong cell and we obtain a distribution between the two cell pairs of 6 AL+1 BL, 4 AL+3 BL or 5 AL+1 BL, 5 AL+3 BL (in Table 2 abbreviated as "$\frac{6}{1}-\frac{4}{3}$ or $\frac{5}{1}-\frac{5}{3}$"), instead of the regular one of 6 AL, 4 AL+4 BL or 5 AL+2 BL, 5 AL+2 BL (abbreviated as "$\frac{6}{0}-\frac{4}{4}$ or $\frac{5}{2}-\frac{5}{2}$"). The most plausible explanation of this type of irregular distribution is disturbance in the pairing and migration during the second division (Plate 2 h). Chromatids originating from univalents, not paired during first metaphase, might be less inclined to pair regularly during the second metaphase and might segregate at random during that phase. With this assumption the result of chromosomal distribution between the two cells of the half-tetrads might depend on the type of pairing in first or second meiotic division.

2. The hybrid 2n=18 (6 AL+12 BL)

The parents of the above-mentioned hybrid have 2n=12 AL and 2n=24 BL chromosomes. All chromosomes from each parent have a size of their own and it is thus possible to recognize the origin of the chromosomes in the hybrid. At first metaphase a complete pairing is reached with six multi-associations (Plates 3 and 4). Incomplete pairing with univalents has, however, been seen, and the cells studied at first metaphase have given the types of pairing associations demonstrated in Table 3.

The results of the observations of the first mitotic division of the pollen tetrads are recorded in Table 4. In the same way as for the above-described hybrid the chromosome patterns of the four cells of the tetrads can be divided up into two equal half-tetrads, with the exception of the three patterns marked with an asterisk in Table 4. Each of these equal pairs of half-tetrads has a total chromosome set

TABLE 3. *Pairing associations observed at first metaphase in two 18-chromosome hybrids of the (6 AL+12 BL)-constitution.*

Associations	Frequencies	
	No. 8	No. 9
6 AL BL_{II}	34	9
5 AL BL_{II}+AL BL+BL	14	20
5 AL BL_{II}+AL+2 BL	—	1
4 AL BL_{II}+2 AL BL+2 BL	2	12
4 AL BL_{II}+AL BL+AL+3 BL	1	1
3 AL BL_{II}+3 AL BL+3 BL	—	7
Total number of cells	51	50

corresponding to that of the somatic one of the studied hybrid, i.e. 6 AL+12 BL. Thus, we also here must have had a morphologically equational first meiotic division giving rise to two equal dyads. In Table 4 the four cells of each tetrad line have been furnished in such an order that the two former and the two latter cells taken together will always form such a half-tetrad. Between the two cells of these half-tetrads the AL-chromosomes seem to have been distributed at random during the second meiotic division. This random distribution can be demonstrated by comparing the observed frequencies of the four possible type of distribution with the expected ones of the random distribution (Table 5). This comparison gives a satisfactory agreement with $0.3 > P > 0.2$.

Also the small BL-chromosome behaves in the same way in the present hybrid as in the one with 14 chromosomes described above. In the majority of the investigated cells, i.e. in 150 of the 212 studied cells (71 per cent), two BL-chromosomes always replace an AL-chromosome. In this way the tetrad cells will normally contain one of the following chromosome patterns: 6 AL, 5 AL+2 BL, 4 AL+4 BL, 3 AL+6 BL, 2 AL+8 BL, 1 AL+10 BL, or 12 BL. In 62 of the 212 investigated cells (29 per cent), one of the small BL-chromosomes has been "mis-located", giving rise to one cell with a supernumerary BL-chromosome and another with a BL in deficit. These two types of cell always occur together in the half-tetrads, indicating that their origin is due to disturbances in distribution during the second meiotic division. In only three of the observed tetrad combinations (marked with an asterisk in Table 4), have one or two of the univalents not split during first meiotic division, but have migrated as a whole unit to one of the poles.

TABLE 4. ***Distribution of large (AL) and small (BL) chromosomes in the pollen tetrads of two 18-chromosome hybrids of the (6 AL+12 BL)-constitution.***

In the first column of the table each line demonstrates the distribution pattern of chromosomes between the four cells of the tetrad. Each group of figures represents one cell, the top figures giving the number of large (AL) chromosomes and the bottom figures the number of small (BL) chromosomes of each of the four cells of the tetrad.

Chromosome distributions	No. of tetrads		Chromosome distributions	No. of tetrads	
	No. 8	No. 9		No. 8	No. 9
AL 4 — 2 — 4 — 2 BL 4 — 8 — 4 — 8	4	3	AL 4 — 2 — 3 — 3 * BL 4 — 7 — 6 — 7	1	—
AL 4 — 2 — 4 — 2 BL 4 — 8 — 3 — 9	1	—	AL 4 — 2 — 3 — 3 * BL 3 — 6 — 7 — 7	1	—
AL 4 — 2 — 4 — 2 BL 4 — 8 — 5 — 7	2	1	AL 5 — 1 — 5 — 1 BL 2 — 10 — 2 — 10	2	1
AL 4 — 2 — 4 — 2 BL 3 — 9 — 5 — 7	—	1	AL 5 — 1 — 5 — 1 BL 2 — 10 — 3 — 9	1	—
AL 4 — 2 — 4 — 2 BL 3 — 9 — 5 — 7	—	1	AL 5 — 1 — 4 — 4 BL 2 — 10 — 2 — 8	1	1
AL 3 — 3 — 3 — 3 BL 6 — 6 — 6 — 6	1	1	AL 5 — 1 — 4 — 2 * BL 3 — 10 — 4 — 7	1	—
AL 3 — 3 — 3 — 3 BL 6 — 6 — 5 — 7	1	—	AL 5 — 1 — 4 — 2 BL 1 — 11 — 4 — 8	—	1
AL 4 — 2 — 3 — 3 BL 4 — 8 — 6 — 6	7	8	AL 5 — 1 — 4 — 2 BL 3 — 9 — 4 — 8	—	1
AL 4 — 2 — 3 — 3 BL 5 — 7 — 6 — 6	1	2	AL 5 — 1 — 4 — 2 BL 2 — 10 — 3 — 9	—	1
AL 4 — 2 — 3 — 3 BL 4 — 8 — 5 — 7	2	1	AL 5 — 1 — 3 — 3 BL 2 — 10 — 7 — 5	—	1
AL 4 — 2 — 3 — 3 BL 3 — 9 — 5 — 7	1	1	AL 6 — 0 — 5 — 1 BL 0 — 12 — 2 — 10	1	—
Total number of tetrads	20	19	Total number of tetrads	8	6

TABLE 5. ***Frequencies of distribution of AL chromosomes on the two cells of the half-tetrads of the (6 AL+12 BL)-hybrids, as compared with the expected values at random distribution.***

Distributions	0:6	1:5	2:4	3:3
Frequencies found	1	16	57	32
Ratio expected	1	6	15	10
Frequencies exp.	3.31	19.87	49.69	33.13

$0.3 > P > 0.2$

3. The hybrid 2n=30 (6 AL+24 CL)

The parents of the present hybrid are the two European species *L. campestris, s. str.* 2n=12 AL and *L. sudetica* 2n=48 CL. Also here the chromosomes of the two parents can easily be recognized in the chromosome pattern of the hybrid. At first metaphase six large multi-associations built on the six AL-chromosomes are easily seen and in addition to these large associations several small CL-univalents are found (Plates 5 g—i and 6 a—d). The small univalents of the CL-chromosomes are usually split during first metaphase and each chromatid migrates to its nearest pole.

The study of the first meiotic metaphase of this hybrid has been difficult to perform on account of its very small CL-chromosomes, nor has it been possible to table the particularities of its pairing configurations of whole cells. For this reason the pollen mitosis of the present hybrid has been studied with special care. From this study it has also been possible to obtain information about the occurrence of pairing and chromatid exchange between the AL- and CL-chromosomes. Concerning the results of crossing-over between these chromosomes the following working hypothesis has been used: If there really occurs a pairing and an exchange between the chromatids of the AL- and the CL-chromosomes during meiosis, then the AL-chromatids must sometimes break into two pieces according to the schedule of Fig. 3 a. Thus, chromosomes of an intermediate size occurring in the pollen mitosis would serve as a verification of the correctness of the hypothesis, i.e. the occurrence of pairing and chromatid exchange between the AL- and the CL-chromosomes.

The results of the observation at first mitotic division of the pollen tetrads of the hybrid studied here is demonstrated in Table 6. Each section of the table represents the description of one investigated tetrad, and each group of figures of the first two columns represents one of the cells of the tetrad. The first column gives the observed number of large AL-, intermediate, and small CL-chromosomes of each of the four cells of the studied tetrad. The top figures give the number of AL-chromosomes, the middle figures the intermediate ones, and the bottom figures the number of CL-chromosomes. The chromosomal distributions between the four cells of the tetrads are very variable and none of the studied tetrads are identical. To obtain some possibility of classifying the cells and the tetrads the figures must, if possible, be adjusted to the occurrence of the original parental chromosomes, i.e. to the AL-

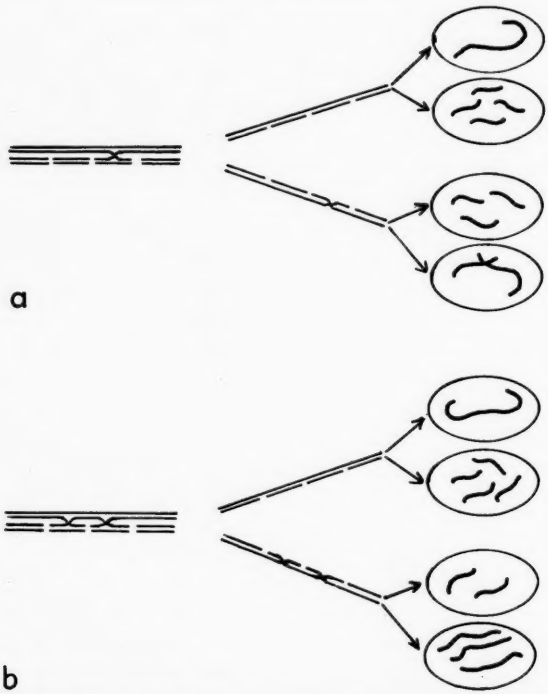


Fig. 3. Hypothetical results of the chromatid exchange between the long AL- and the short CL-chromatids in the (6 AL+24 CL)-hybrid. (Only the possibilities discussed in the text are included in the drawings.) a: indicates the results of one chromatid exchange between the AL-chromatid and one of the proximal CL-chromatids; b: indicates the result of a double chromatid exchange between one AL-chromatid and two proximal CL-chromatids. For further explanation see text.

and the CL-chromosomes. According to the schedule of Fig. 3 a it is postulated that two intermediate chromosomes correspond to one AL- and one CL-chromosome. Moreover, the "whole chromatids" usually seem to migrate to one and the same pole independently of the fact that they might be based on more than one unit. Consequently, the intermediate chromosomes ought to be present in pairs in the cells, a phenomenon which has been observed in the majority of the studied cells. In some tetrad cells, where these pairs do not occur, the cause must be due to some kind of double crossing-over, an illustration of which is demonstrated in Fig. 3 b. The occurrence of such double crossing-overs must also have rendered the distinguishing between the intermediate and the

TABLE 6. *Distribution of large (AL), intermediate (Int.), and small (CL) chromosomes on the four cells of the pollen tetrads in the hybrid L. campestris × L. sudetica (2n=6 AL+24 CL).*

In the table each section refers to one tetrad. In the first column, giving the results of the observations, each group of figures of the sections represents one cell of the tetrad; the top figures give the numbers of large chromosomes, the middle figures the numbers of intermediate chromosomes, and the bottom figures the small chromosomes. (For further explanations see text.)

Chromosomal distribution observed in tetrads					"Adjusted distribution"				Totals of chromosome units in half and whole tetrads
AL	3	2	2	3	3	3	3	3	
Int.	0	2	2	0					30+30=60
CL	12	11	12	11	12	12	13	11	
AL	2	2	1	1	3	3			
Int.	0	2	3	5					30+30=60
CL	14	9	12	8	14	10			
AL	3	2	3	1	3	3	4	2	
Int.	0	2	2	2					30+30=60
CL	11	12	10	12	11	13	11	13	
AL	3	2	3	1	3	3	4	2	
Int.	0	2	2	2					30+30=60
CL	11	12	10	12	11	13	11	13	
AL	3	2	3	1	3	3	4	2	
Int.	0	2	2	2					31+29=60
CL	13	11	9	12	13	12	10	13	
AL	1	1	2	1	3	3	4	2	
Int.	4	4	4	2					29+31=60
CL	9	10	8	14	11	12	10	15	
AL	2	3	3	2	3	3	4	2	
Int.	2	2	2	0					29+31=60
CL	10	10	8	16	11	12	9	16	
AL	1	1	2	2	3	3	4	2	
Int.	4	4	4	0					26+34=60
CL	8	8	10	16	10	10	12	16	
AL	3	2	1	3	3	3	2	4	
Int.	0	2	2	2					26+34=60
CL	10	9	12	14	10	10	13	15	

Chromosomal distribution observed in tetrads					"Adjusted distribution"				Totals of chromosome units in half and whole tetrads
AL	2	1	3	1	4	2	4	2	29+31=60
Int.	4	2	3	2					
CL	7	13	8	14	9	14	10	15	
AL	4	2	2	1	4	2	4	2	30+30=60
Int.	0	0	4	2					
CL	11	13	10	11	11	13	12	12	
AL	3	1	4	2	4	2	4	2	30+30=60
Int.	2	2	0	0					
CL	8	14	10	14	9	15	10	14	
AL	4	1	4	1	5	1	5	1	30+30=60
Int.	2	0	2	0					
CL	8	15	8	15	9	15	9	15	
AL	5	0	4	1	5	1	5	1	26+34=60
Int.	0	2	3	0					
CL	7	12	7	19	7	13	9	19	
AL	4	0	3	2	5	1	3	3	29+31=60
Int.	2	3	0	2					
CL	6	14	12	12	7	16	12	13	
AL	5	1	4	2	5	1	4	2	30+30=60
Int.	0	0	0	0					
CL	7	17	12	12	7	17	12	12	
AL	5	1	3	2	5	1	4	2	30+30=60
Int.	0	0	2	0					
CL	5	19	5	18	5	19	6	18	
AL	3	1	4	0	5	1	4	2	31+29=60
Int.	4	0	0	4					
CL	6	17	13	8	8	17	13	10	
AL	4	1	3	2	5	1	4	2	28+32=60
Int.	2	0	2	1					
CL	6	15	11	13	7	15	12	14	
AL	0	5	2	3	0	6	3	3	26+32=58
Int.	0	2	2	0					
CL	16	3	15	10	16	4	16	10	

TABLE 7. *Frequencies of distribution of AL-chromosomes on the cells of the half-tetrads in the pollen of the (6 AL+24 CL)-hybrid, as compared with the expected values at random distribution.*

Distributions	0:6	1:5	2:4	3:3
Frequencies found	1	7	17	11
Ratio expected	1	6	15	10
Frequencies exp.	1.22	7.31	18.28	12.19

$0.9 > P > 0.8$

small CL-chromosomes very difficult, thus, explaining the difficulty of classifying the chromosomes in many cells. If we adjust the figures of the first column of Table 6 according to the assumptions of Figs. 3 a and b by replacing two intermediate chromosomes by one AL- and one CL-chromosome or three intermediates by one AL and two CL, we receive the adjusted chromosomal distribution of the second column. If we now study the adjusted distribution of the AL-chromosomes (the top figures of each square of the second column of Table 6), we find that the total number of AL-chromosomes of two of the four cells is always six, thus, indicating the morphologically equational first meiotic division. The four cells of the tetrads of Table 6 have been furnished in such an order that the two former and the two latter are assumed to belong together to a half-tetrad. The six AL-chromosomes of the dyad are during the second meiotic division distributed at random between the two cells of the half-tetrad. All four possible kinds of distribution have been observed among the studied half-tetrads, i.e. 0:6, 1:5, 2:4, and 3:3. That these distributions really occur at random can be shown by testing the frequencies of the four distribution possibilities with the expected values of random distribution. In Table 7 these frequencies have been compared and the agreement is good ($0.9 > P > 0.8$)

The distribution of the small CL-chromosomes has not been so accurate as the one of the large AL-chromosomes. The two half-tetrads very often have 24 CL-chromosomes each, thus bringing the total number of chromosomes in each half-tetrad up to 30 (6 AL+24 CL). But sometimes the total chromosome numbers of the half-tetrads are 29 and 31, and the difference between the chromosome numbers of the half-tetrads may grow as large as 26 to 34 (cf. Table 6 column 3). But it has always been only the small CL-chromosomes which have been distributed irregularly, not the "AL-chromosomes". At first metaphase a fairly high number of CL-univalents are formed causing a certain irregularity of meiosis. However, the univalents usually split evenly during this phase,

and thus their presence cannot be the main cause of the very uneven distribution of the CL-chromatids between the half-tetrads. A more likely explanation of these disturbances may be the chromatid exchanges that take place in the proximally paired CL-chromosomes. From this phenomenon we obtain a single CL-chromatid, which has to be distributed at random during the first meiotic division.

The third column of Table 6 gives the total number of chromosome bodies found in the four cells of the studied tetrads. Independently of the occurrence of crossing-over, the total number of chromosome units should be 60. In the majority of the examined tetrads (18 tetrads) the total number is 60. But in some of them only 59 (5 tetrads) or 58 (6 tetrads) have been found. (Only one of these "incomplete" tetrads has been tabled.) In the last-mentioned tetrads one small CL-chromosome may have been lost in the first or second meiotic divisions. It is, however, also possible that the missing chromosomes have not been observed during the examination of the cells. One or two of the small chromosomes might easily stick together with one of the other chromosomes.

IV. COURSE OF MEIOSIS IN LUZULA CAMPESTRIS

From the tetrad analysis of the three *Luzula* hybrids, we have learned the following particularities concerning the course of meiosis in *L. campestris*. Firstly, the first meiotic division ought to be cytologically equational and the second one reductional; secondly, the small chromosomes of the endonuclear polyploids are homologous to their corresponding parts in the large ones of the 12-chromosomal diploid; and thirdly, chromatid exchange may occur between homologous chromatids during meiosis. These three characteristics will be used in our further investigations and discussions of meiosis in *Luzula*.

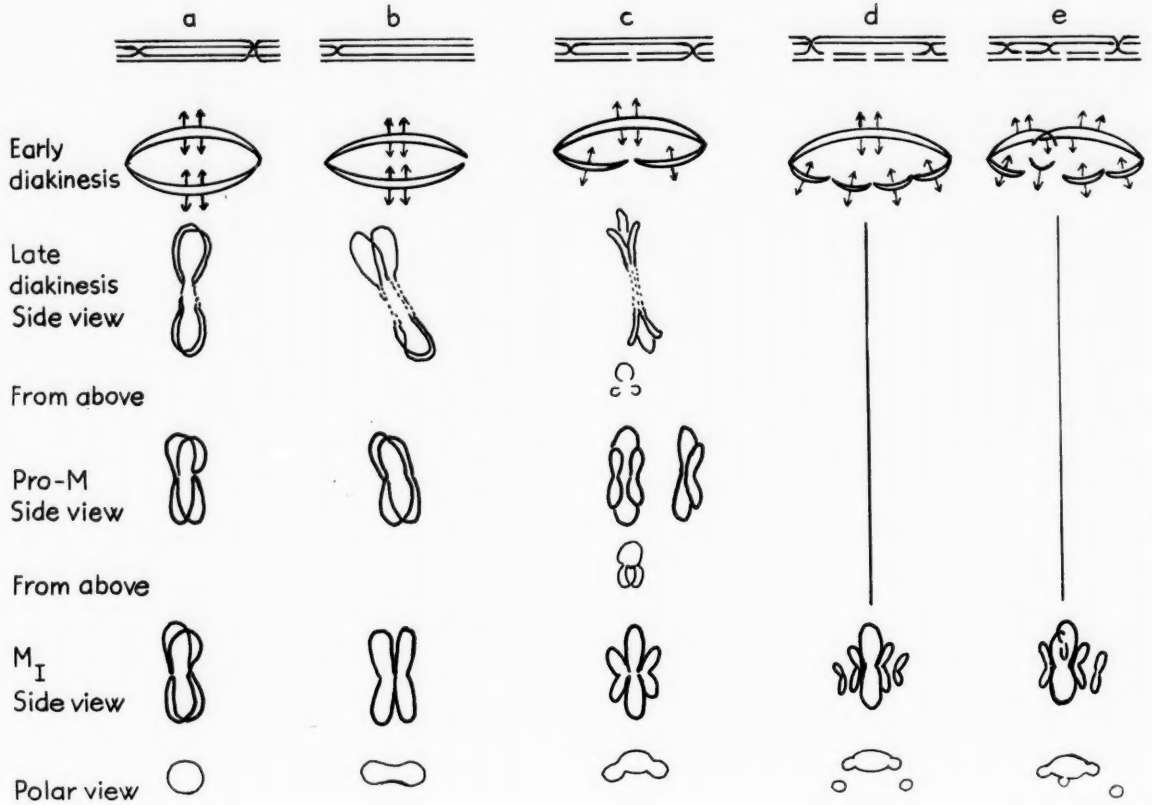
In order to obtain a general survey of the formation of the meiotic configurations found in the studied hybrids of *L. campestris* and a better understanding of the origin of the chromosomal distribution between the four cells of their tetrads the schedule of Fig. 4 has been made. The development of the three investigated types of association is demonstrated in the schedule, and an attempt is made to interpret the characteristically shaped configurations of first metaphase. When making the drawings of the earlier phases in side view the chromatids have been used to explain the postulated structure of the configurations. This structure has been built up mainly from the cytological appearance of

the AL BL_{II} associations at late pro- and early metaphase, the ones of AL_{II} and AL CL_{IV} having been assumed to have an analogous development. The associations shown from above and the ones demonstrating the later meiotic stages (A_I—tetrad) have been illustrated by the outlines of the observed configurations. This schedule has been used as a working hypothesis for interpreting the observed meiotic configurations and it will be followed when studying the development of the associations of the illustrated cells (Plates 1—6).

The meiosis of the 12-chromosomal *L. campestris* with all chromosomes of the AL-size has only been given superficial study. This species has been cytologically investigated several times earlier, especially thoroughly by BROWN (1954). Early meiotic prophase has not been studied in the present material but previous investigators have demonstrated pairing of homologous chromosomes and the formation of chiasmata. At late prophase six pairs of chromosomes are formed (Plate 1 a), and at late diakinesis a doubleness of the associations appears. This doubleness is manifested in the dumbbell-like shape of the bivalents almost as "two chromosomes in juxtaposition" (Plate 1 a and b).

At early metaphase the bivalents are strongly contracted and densely pressed together in a firm spindle. Six chromosome bodies can usually be counted during this stage. More or less "unpaired bivalents" are, however, not uncommon (Plate 1 c). In side view the metaphasic configurations appear as pairs of rods but later on the pairs usually achieve a ring-shaped appearance (Plate 1 c and d, Plate 2 b, c, e, f; Figs. 4 a—b). At this stage the four chromatids can be discerned in the associations, as already described from previous investigations of *Luzula* by MALHEIROS, DE CASTRO, and CAMARA (1947) and BROWN (1954). This same picture is also obtained in other material with diffuse centromeres as, for instance, in the *Coccids* studied by HUGHES-SCHRADER (1948). In polar view the bivalents are found as rounded or rod-shaped bodies, a difference in appearance of the configurations which is assumed to depend on the number and localisation of chiasmata formed in the associations. In the former type of bivalent chiasmata are supposed to occur on both sides of the intermediate region, in the latter type only one chiasma is assumed to have been formed (Figs. 4 a and b). In the cell of Plate 2 a both closed and open bivalents must occur among the four homomorphic associations, as only two round bodies are found.

During late anaphase and early telophase the two homologous chromatids are seen to stick close together, but during interphase they be-



a and b (cont.)	c (cont.)	d (cont.)	e (cont.)

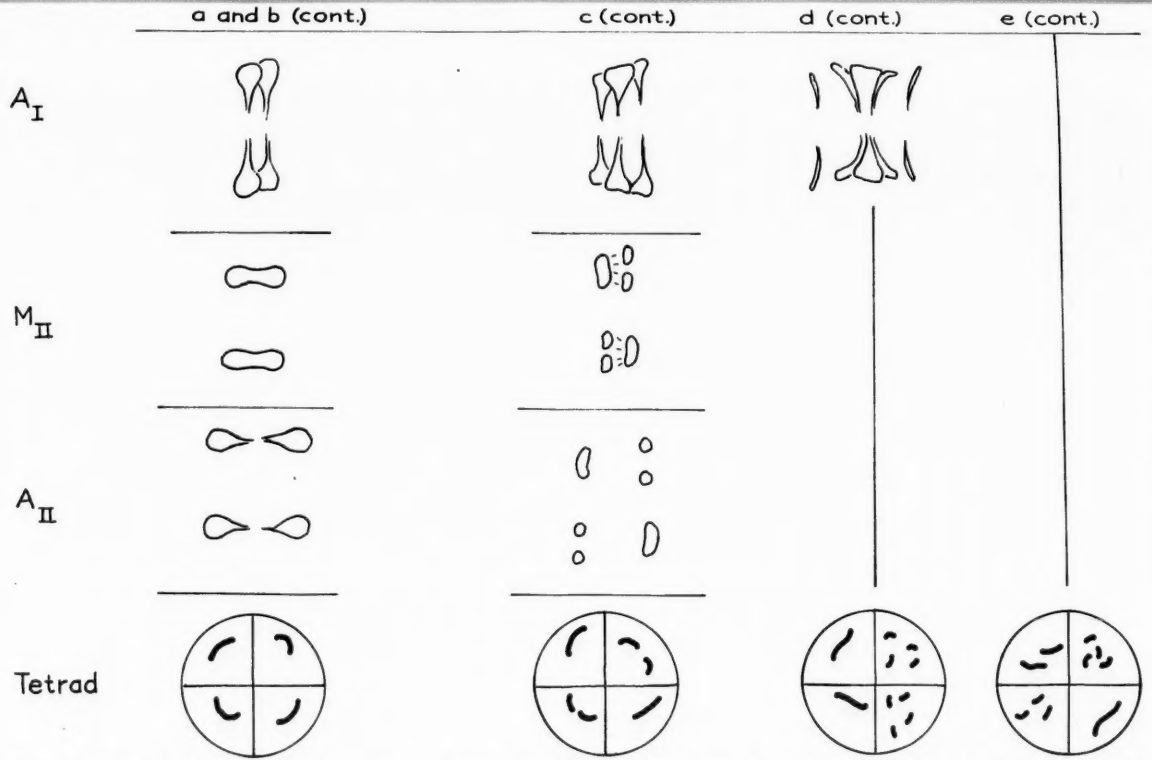


Fig. 4. Course of meiosis in *Luzula campestris* demonstrated by the three types of meiotic associations studied, with special regard to the formation of the metaphase associations. a: the homomorphic bivalent of AL_{II} wiht chiasmata on both sides of the terminalisation zone; b: the homomorphic bivalent AL_{II} with chiasmata only on one side of the terminalisation zone; c: the heteromorphic association of AL BL_{II} with chiasmata on both sides of the terminalisation zone; d: the heteromorphic association of AL CL_{IV} with chromatid exchanges only between the two terminal CL-chromatids and their homologous parts of the AL-chromatids; e: the heteromorphic association of AL CL_{IV} with chromatid exchanges also between one of the proximal CL-chromatids and its homologous part of an AL-chromatid. For further explanation see the text.

come more or less diffuse and difficult to study. At early second metaphase two firm spindles are seen, each with six strongly contracted and densely fixed chromosome bodies (Plate 1 e—f). The chromatids are now again paired, being similar to the ones of the first metaphase. In polar view the chromosome bodies are, however, smaller than the ones of first metaphase and in side view each chromatid pair of the second metaphase looks like half a bivalent of the first metaphase. Thus, it seems as if neither the whole meiotic cell nor the meiotic chromosomes have been enlarged during the first division (cf. Plate 1 c and f). The same size differences between the chromosomes of the two meiotic divisions have been demonstrated in other material with diffuse centromeres as, for instance, in the *Lepidoptera* (FEDERLEY, 1945; SUOMALINEN, 1953). At late metaphase and early anaphase of the second division the spindle becomes more dispersed in the same way as during the first division. It is now possible to observe the paired chromatids separating and migrating to the poles.

The meiosis of the endonuclear polyploids follows the same course as the one of the 12-chromosomal diploid. The bivalents are merely somewhat smaller and multivalents have never been observed. In Plate 1 j—k the meiosis of the endonuclear tetraploid is shown. Plate 1 j gives the early dense stage of the first metaphase and Plate 1 k a later stage with more dispersed bivalents. Plate 1 i gives first metaphase of the 16-chromosomal strain of *L. campestris* with 2n=8 AL+8 BL. Here it is found that it might be difficult to distinguish between the AL- and BL-bivalents. The shape of the bivalents is the same and the size difference is best shown in the lighter staining of the smaller BL-bivalents.

We have studied two different hybrids containing both AL- and BL-chromosomes, one with 2n=10 AL+4 BL and the other one with 2n=6 AL+12 BL. The first-mentioned hybrid has four homomorphic AL-bivalents and two heteromorphic AL BL_{II} associations, whereas the second one has all chromosome association heteromorphic of the AL BL_{II} type. Early prophase has not been studied in the present hybrids. At late prophase six chromosome associations are usually found in both hybrids, and at late diakinesis these associations achieve their dumbbell-like shape. At this stage the difference between the homo- and heteromorphic associations comes into sight. In the former ones the two halves of the association usually acquire a rounded outline (Plate 1 b, Figs. 4 a—b), sometimes with a hole in the middle (Plate 1 b, the bivalent to the right), but in some aspects the halves might give the appearance of a double body (Plate 1 a, the top bivalent; Plate 1 b, the

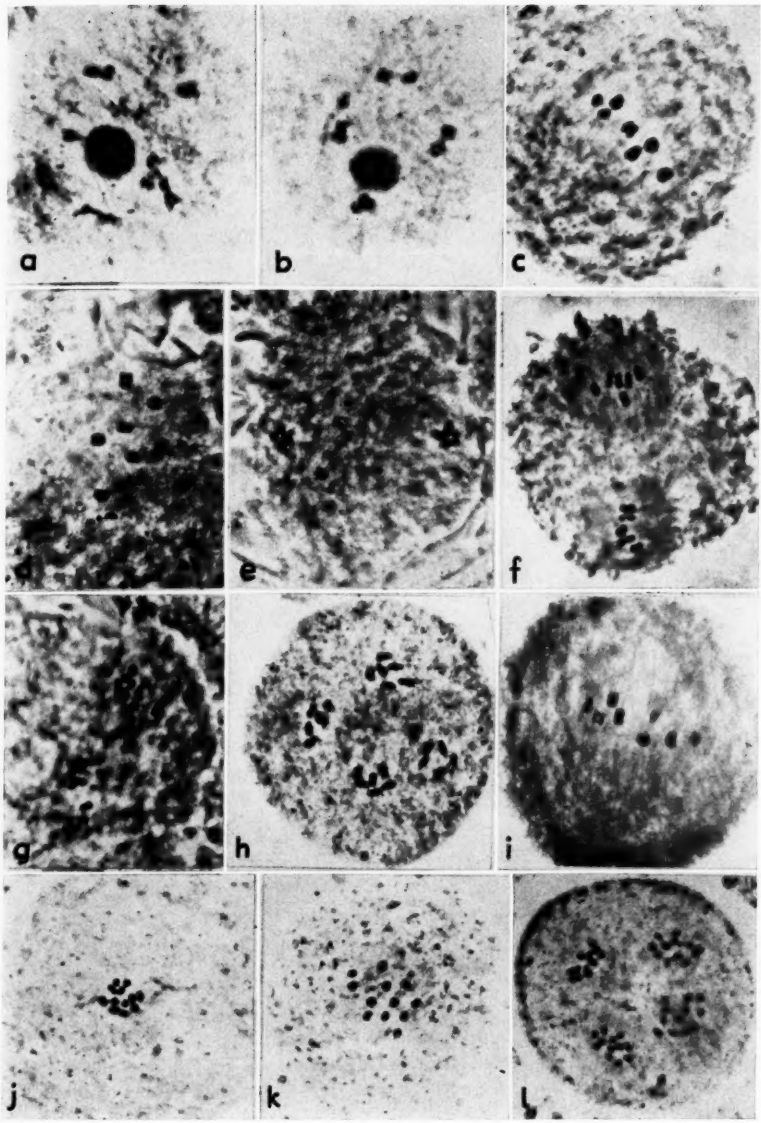


Plate 1. Meiosis and pollen tetrads of a—i: *L. campestris* with 2n=12 and 2n=16, j—l: *"L. australasica"* with 2n=24 (BL). a—b: late diakinesis, cell b demonstrates the dumbbell-shaped bivalents; c: early metaphase I; d: late metaphase I; e: early metaphase II, polar view; f: metaphase II, side view; g: anaphase II; h: first mitosis of the pollen tetrad; i: metaphase I of the 16-chromosome (8 AL+8 BL) strain; j: early metaphase I; k: metaphase I; l: first mitosis of the pollen tetrad. — × 1,200.

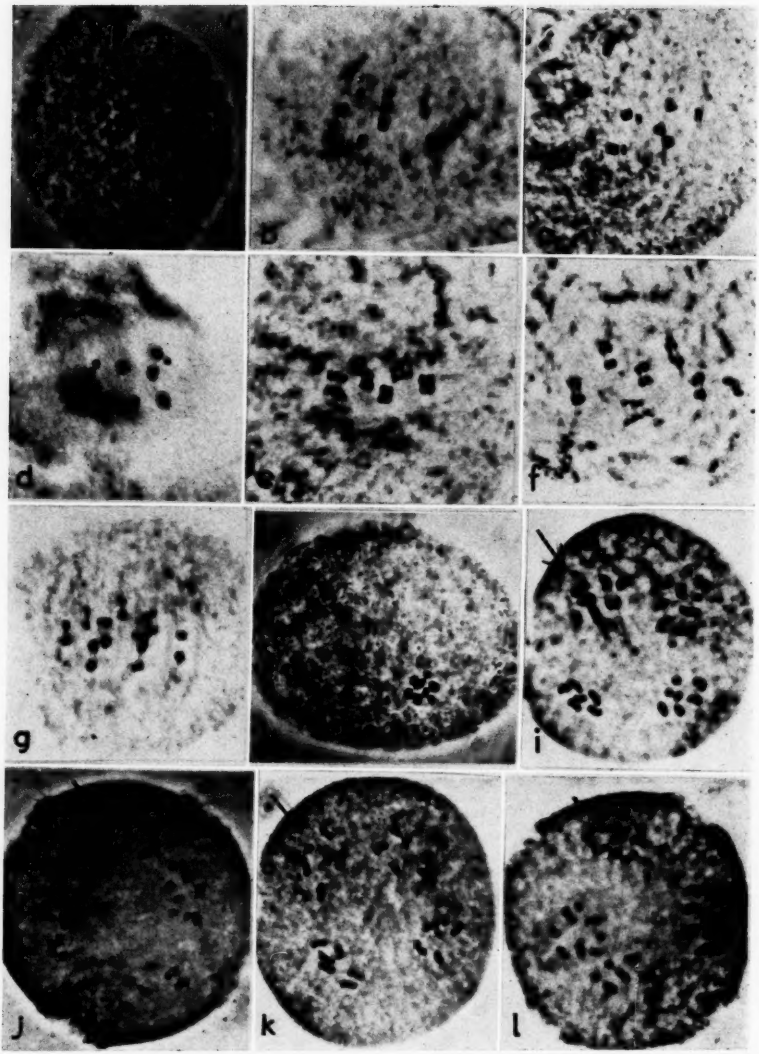


Plate 2. Meiosis and pollen tetrads of the hybrid 2n=10 AL+4 BL. a: early metaphase I, polar view; b—c: metaphase I, side view of the chromosome associations; d: metaphase I, polar view. In the two heteromorphic associations one of the BL-chromosomes is released in the same way as shown in side view in c. e—f: early anaphase I associations in side view. The two-ring associations of the two heteromorphic "bivalents" are shown. The four homomorphic ones form a single ring configuration. g: anaphase I, separation of associations paired in the same way as in cell b; note the similarity between univalents and some of the bivalents. h: metaphase II with irregular pairing; i—l: first mitotic metaphases of the pollen tetrads. The metaphase plates show (starting at the arrows): i: 5 AL+2 BL, 6 AL+1 BL, 4 AL+3 BL and 5 AL+2 BL; j: 5 AL+2 BL, 4 AL+4 BL, 6 AL and 5 AL+2 BL; k: 4 AL+4 BL, 6 AL, 4 AL+4 BL and 6 AL; l: 5 AL+1 BL, 5 AL+2 BL, 5 AL+2 BL and 5 AL+3 BL. — × 1,200.

bivalent at the bottom). In the latter type of bivalent the two halves of the associations are characteristically divided into three tongues or branches (Fig. 4 c, late diakinesis), most easily seen in side view. In the cells of Plate 3 b—e these configurations are marked with single arrows. From above, these trisected branches show one big and two small bodies marked with double arrows in the cells of Plate 3 e—g. This triplicity of the two main parts of the configuration indicate the possibility of each half of the association being composed of one large AL- and two small BL-chromatids — the result of an early "equational" separation of the original prophasic association. Note especially the "bivalent" below to the right in Fig. e of Plate 3, where the pairing of the large AL- and the two small BL-chromatids are illustrated by the two identical halves of the "bivalent". It has not been possible to investigate cytologically how the connections between the two parts of the configurations are made up. These connections might be residuals of chiasmata or they could be the terminal points of the chromosomes which still at this stage keep together. Both these phenomena probably cooperate.

There is only one of the chromosome associations of the cells which can always be recognized with certainty and in which the orientation of the chromatids might be determined, and that is the nucleolus-attached one. At the early meiotic prophase of *Luzula* one of the ends of this bivalent is always connected with the nucleolus (BROWN, 1954; KUSANAGI and TANAKA, 1960). In the cells of late diakinesis, when the associations have reached their dumbbell-shape, it has always been found to be the connection between the two halves which at this stage is attached to the nucleolus (Plate 3 e). These circumstances indicate that at least one end of the nucleolus-attached chromatids must be situated at the connection between the two halves, and that there must be some kind of junction between the ends of the four chromatids at that point. In other words, the ends connecting each other must be homologous as they ought to constitute the nucleolus-attached point of the chromatids. If the other ends of the bivalent are held together in the same way, we obtain a configuration similar to the one illustrated in Fig. 4 a—c at late diakinesis.

However, when passing through the later stages of prophase the chromosome associations become more condensed, and during that time the two halves of the associations approach each other (cf. Plate 3 a—c and g). At this stage the breaking down and disappearance of nucleolus occur and simultaneously the chromosome associations acquire a fairly diffuse appearance (Plate 3 h). At pro-metaphase the

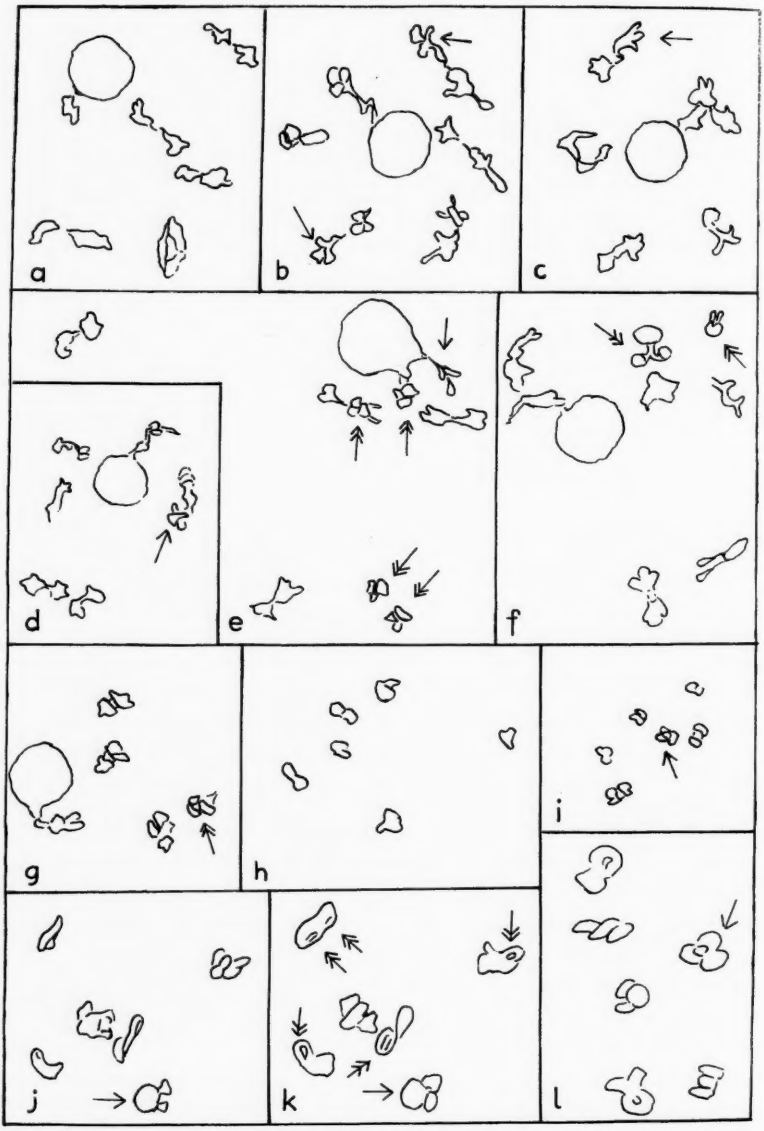
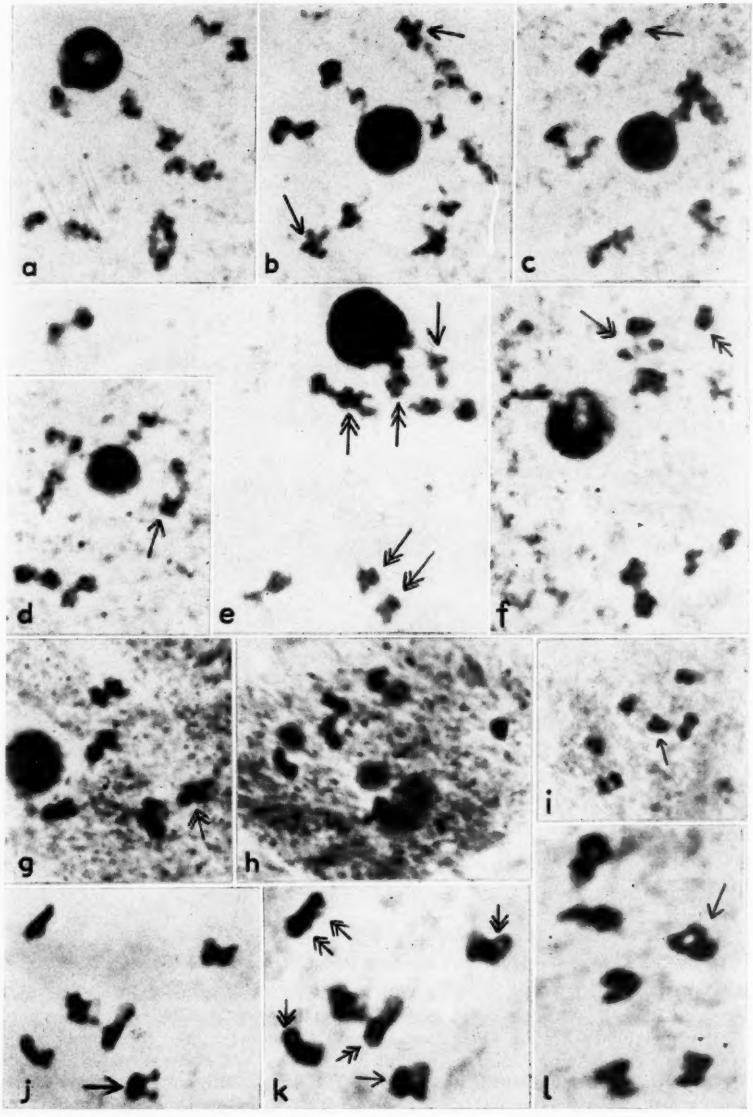


Plate 3. Diakinesis and prometaphase of the hybrid 2n=6 AL+12 BL. a: commencing condensation of the associations at diakinesis, the "bivalent" at the bottom still forms a ring; b—e: the simple arrows demonstrate the triplicity of the ends of the associations found in side view; e—g: the double arrows show the triplicity of the two ends of the associations from above; g—i: the two ends of the dumbbell approach each other; g: the triplicity can still be seen in one of the associations; h: the nucleoli are breaking down and disappearing, and the chromosomes are difficult to distinguish:



i: the ring shape of the associations can be seen from above whereas the two parts of the associations in side view still are at a certain distance; j—l: pro-metaphase with six associations; j—k: the same cell at different optic levels. The double arrows point at the associations, in which the ring shaped chromatids are visible, the single arrows at the associations in which the ring structure of the whole configuration can be seen. — e: × 2,500; j—l: × 3,200; the other pictures: × 1,800.

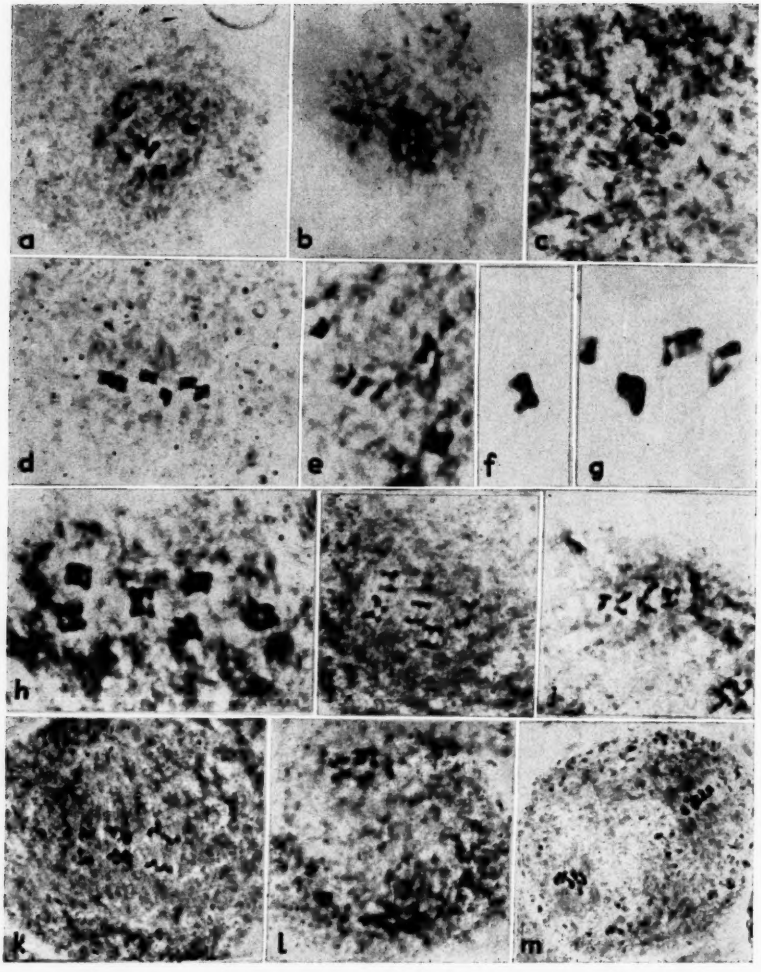


Plate 4. Meiosis of the hybrid 2n=6 AL+12 BL (cont.); a: prometaphase, the same cell as in Plate 3 j—k at a lower magnification; b: early metaphase I, polar view; c: early metaphase I, side view; d: metaphase I, side view, the three rods of the associations can be seen; e: larger magnification of a three-rod association; f: optic section through the middle rod showing the eight-shaped chromatids; g: optic section through one of the side rods showing the askew shape of the chromatid, part of the same cell as in d at a larger magnification; h: large magnification of a late metaphase I showing the three rods of the associations. The middle one shows the six chromatids; i—k: separation of the associations at anaphase I; l: interkinesis, in the upper nucleus the doubleness of the chromosome bodies can be distinguished; m: metaphase II, one "univalent" can be seen in each plate. — e—h: × 2,500; a and m: × 1,000; the other pictures: × 1,200.

bivalents are again distinct. At this stage each chromatid seems to form a ring (Plate 3 j—l, Fig. 4 c). At certain optic sections through the associations these chromatid rings can be seen. In the cells of Plate 3 j—l such configurations have been marked with double arrows. Secondarily the ring-shaped chromatids seem to be arranged in a more or less closed-ring association as illustrated in Fig. 4 c (pro-metaphase, from above). In the cells of Plate 3 i—l the bivalents revealing this configuration are marked with simple arrows. At the time for orientation of the bivalents in the metaphasic plane the ring associations have opend up. We now have the metaphasic configurations shown in the cells of Plate 4 d—h, the interpretation of which is illustrated in Fig. 4 c at M_I. In side view these associations look like three parallel rods densely pressed together and arranged perpendicularly against the metaphasic plane (Plate 4 e). But also at this stage the chromatids can be seen at certain optic sections. Each rod of the association is composed of two rings, the ones of the central rod are symmetric, together forming a figure of eight (Plate 4 f), whereas the ones of the two side rods are askew (Plate 4 g) and bent up against each other, demonstrating a remnant of the previous ring association. In polar view the six associations at first metaphase are always open, giving the appearance of six somewhat curved rods (Plate 4 b, Fig. 4 c). In this view of early first metaphase it is again difficult to distinguish between the two kinds of association (Plates 2 a and 4 b), i.e. to separate the heteromorphic association from the open homomorphic one. At the transition from metaphase to anaphase a two-ring configuration is seen when the association is observed in side view (Plate 4 h—i, Plate 2 e—g, Fig. 4 c). This double-ring formation separates in the same way as the simple ring formation of the normal AL-bivalents (Plate 2 e—g and Plate 4 h—k, Figs. 4 a—b and c). Six chromatids can be discerned in these heteromorphic associations (Plate 4 h), even if the three chromatids migrating to the same pole usually seem to hold together as one unit (Plate 4 i—k).

During telophase and interphase the chromosomes are difficult to distinguish but a certain doubleness of the chromosome bodies similar to the one of the pro-metaphase may be seen (Plate 4 l). At early second metaphase six densely fixed bodies can be observed in each of the two spindles of the dyad (Plate 4 m and Plate 5 a). When the metaphase turns over into anaphase the spindle becomes less firm and the shape of the chromosome associations can be studied (Plate 5 a—c). It is now possible to distinguish the configurations of the heteromorphic associations (Plate 5 b, Fig. 4 c). At this stage the chromatids are again

paired according to homology with two small BL-chromatids alongside the large AL-ones. At early second anaphase the separation between the AL-chromatids and the two small BL-ones can easily be studied (Plate 5 d—e, Fig. 4 c). This configuration explains the fact that two small BL-chromosomes always replace one of the large AL-chromosomes. Sometimes it has been possible to observe "univalents" during the second meta- and anaphase (Plate 4 m). The occurrence of such "univalents" will explain the random distribution of some of the BL-chromosomes at the second division.

The hybrid to be studied last is the one with 2n=6 AL+24 CL. The only meiotic stage which could be studied in this hybrid is the metaphase of the first division. Here it is possible to distinguish the six large multi-associations and the sometimes numerous small CL-univalents (Plate 5 g—i, Plate 6 a—d). At a closer investigation of these metaphasic configurations, in plates which have not been flattened down too much at smearing (Plate 5 g—i, Plate 6 a, c), it has been found that the large associations are very often surrounded by two small univalents. It seems as if the pairing between the large AL-chromosome and the four small CL-chromosomes at an early stage has been more complete but that at least some of the small CL-chromosomes are almost regularly released at the later stages of metaphase, subsequently appearing as free univalents in the vicinity of the large associations.

In order to find out the structure of the multi-associations of AL CL_{IV}-type, a meiotic development analogous to the one described above for AL BL_{II} has been deduced for the first-mentioned type of association as illustrated in Fig. 4 (d—e). In Fig. 4 d we have such an association with chromatid exchanges between the two terminal CL-chromatids and their homologous parts of the AL-chromatid. In Fig. 4 e chromatid exchanges also occur between the proximal CL-chromatids and their homologous parts of the AL-chromatids resulting in a "breakage" of the AL-chromatid concerned. At early metaphase we obtain in the illustrated associations five or four parallel rods arranged perpendicularly against the metaphasic plane and forming a more or less closed ring. But in later stages two of the rods originating from the proximally paired CL-chromosomes are released and appear as univalents in the vicinity of the large association. In polar view we ought, thus, to find a curved multi-association with two small CL-univalents close at hand (Figs. 4 d and e, M_I polar view). Plate 5 g—i present photos of that stage. The associations of Plate 5 g seem to give the earliest stage. The

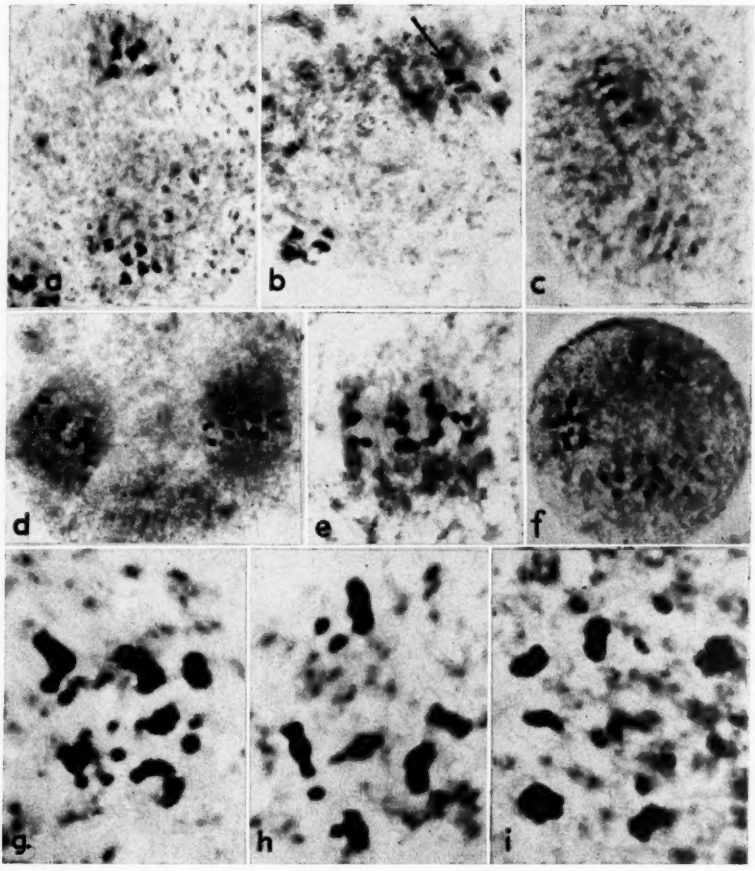


Plate 5. a—e: meiosis of the hybrid 2n=6 AL+12 BL (cont.); a: metaphase II; b: enlargement of an association of one AL- and two BL-chromatids at metaphase II; c: late metaphase II; d: anaphase II, the associated chromatids separating; e: one of the spindles of d enlarged; f: chromosome distribution in a tetrad of the hybrid 2n=6 AL+12 BL showing (in the three lower mitotic plates): 4 AL+4 BL, 3 AL+6 BL, and 2 AL+8 BL; g—i: early metaphase I of the hybrid 2n=6 AL+24 CL, polar view. Six multi-associations and several small univalents can be seen. (For further explanation see the text p. 230.) — g—i: × 3,200; b and e: × 1,900; the other pictures: × 1,200.

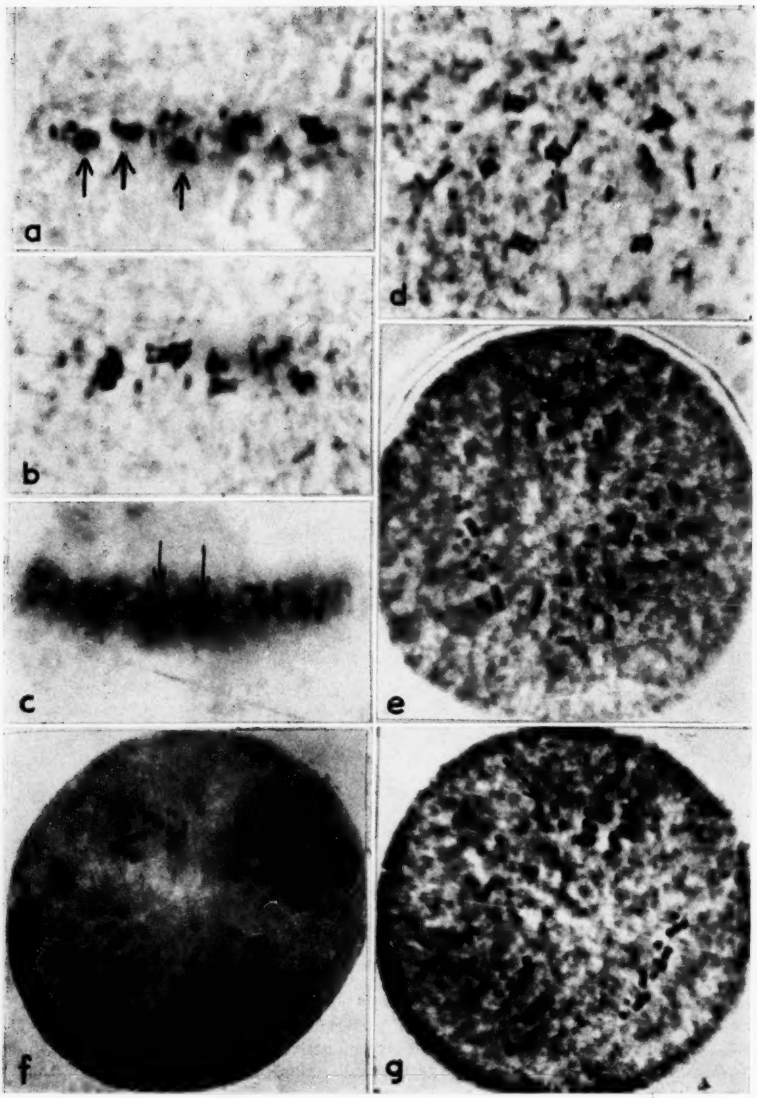
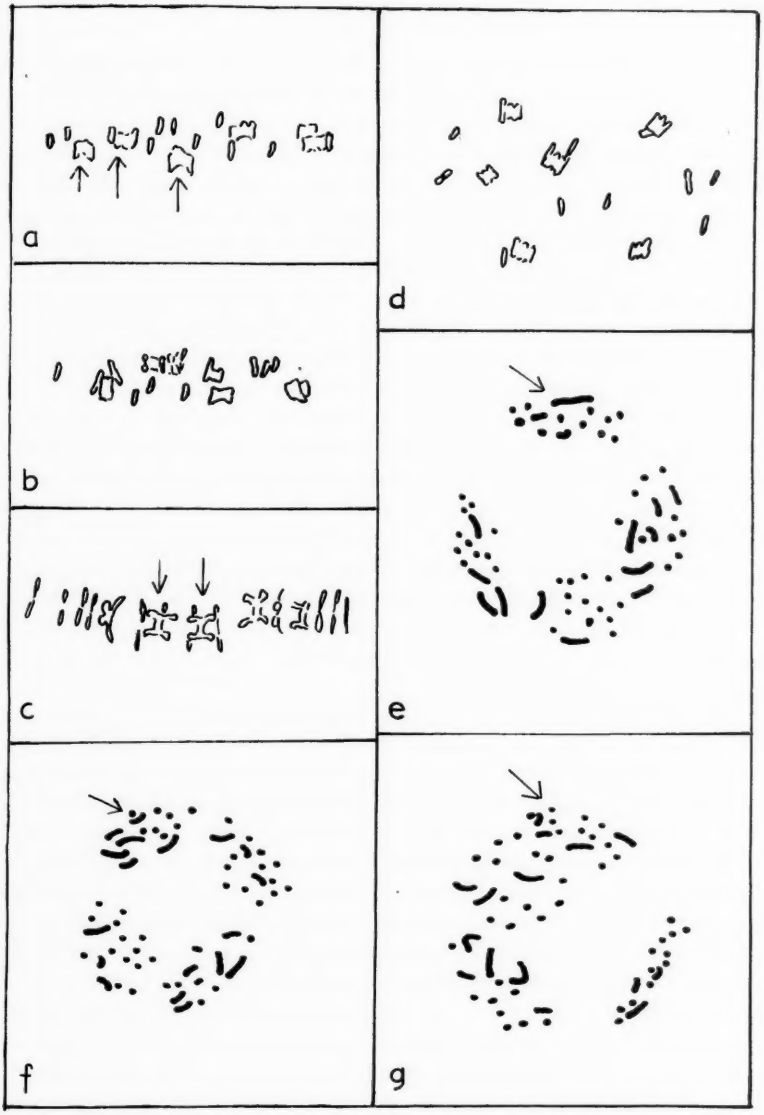


Plate 6. Meiosis and pollen tetrads of the hybrid 2n=6 AL+24 CL (cont.); a—d: metaphase I; a—b: side view of the metaphase plate only slightly flattened. The arrows point to the still curved multi-associations. b: an association forming a multi-ring configuration; c: plate in side viwe of an advanced metaphase I only slightly flattened. The arrows point at the configurations in focus where the two small CL-univalents are found in the vicinity of the main configuration; d: Metaphase plate more heavily flattened down. Six multi-associations are seen and in addition several small



CL-univalents scattered around. e—g: first mitotic metaphases of pollen tetrads. The metaphase plates demonstrate the following chromosome patterns (starting at the arrows): e: 1 AL+2 Int.+11 CL, 2 AL+4 Int.+10 CL, 2 AL+13 CL, 4 AL+11 CL; f: 3 AL+3 Int.+8 CL, 1 AL+2 Int.+13 CL, 2 AL+4 Int.+7 CL, 1 AL+2 Int.+14 CL; g: 2 AL+2 Int.+11 CL, 1 AL+2 Int.+12 CL, 3 AL+2 Int.+9 CL, 3 AL+13 CL. — × 1,900.

six multi-associations are easily found. The top one to the left has its two CL-univalents still stuck to the main body. From the top one in the centre both the CL-univalents are released, and it is possible to observe the tracks along which they have been removed, probably at smearing. The multi-association at the bottom to the right has both its CL-univalents enclosed in the main configuration in the same way as the one described above, and the bottom one to the left is surrounded by four univalents, two of which show their tracks to their main association. In Plate 5 h two of the six multi-associations are out of focus. Close to the top one, its two released CL-univalents are found. Plate 5 i gives the same picture but in a somewhat more advanced stage. Six multi-associations are found; close to the one at the bottom to the left, its two CL-univalents are seen. The other free univalents occur still in pairs, but they seem to have been removed by smearing, on account of which it is not possible to decide to which of the large associations each pair belongs.

In Fig. 4 e at first metaphase it seems theoretically possible to obtain a small univalent-like bud attached to the curved multi-association. Such a body also seems to occur in the cell of Plate 5 h attached to the large association at the bottom. However, it is of course very difficult to distinguish with certainty between such a "bud-univalent" and the regularly released ones.

The early first anaphase illustrated in Fig. 4 d is found in Plate 6 c. Here it is seen that the univalents separate evenly and usually earlier than the large multi-associations. At the bottom of the two columns of d and e of Fig. 4 two likely results of the chromosome distributions between the four tetrad cells are found.

This last-studied hybrid with its AL CL_{IV}-associations is the only one in which chromatid exchange really can be proved cytologically. But if such a chromatid exchange occurs in this hybrid between two fairly distinct species (*Luzula campestris s. str.* and *L. sudetica*), it also ought to occur in the two other hybrids studied here, one of which is a cross between two strains of the same species, the other between two closely related species. And most likely, it is here, as in material with localized centromeres, the residuals of the terminalised chiasmata which help to hold the chromatids of the associations together at first metaphase. To observe cytologically how these chiasmata are arranged and in what way they are terminalised has not been possible in the present material. It has only been possible to trace the course of chiasma terminalisation and chromatid separation from the configurations observed

during later stages. From that study it has been considered most likely that the separation occurs in connection with the terminalisation of chiasmata. Probably the separation and terminalisation start in the intermediate region of the associations. If such a terminalisation of chiasmata is assumed to occur in the homomorphic as well as in the heteromorphic associations, the latter will regularly open up, whereas the former will keep closed if they have at least one chiasma on each side of the terminalisation zone (Figs. 4 a—c). Moreover, in the associations of the AL CL_{IV}-type the two CL-chromosomes with the proximal pairing position in the association will always become completely released from the large association after such a terminalisation (Figs. 4 d and e). The two CL-chromosomes with the terminal pairing position in the association will become released only when they do not have any chiasmata with their AL-partner. Thus, after complete pairing in the AL CL_{IV}-association at least two small CL-univalents will surround the large chromosome body (Plate 5 g—i, Plate 6 c).

The present study has been carried out in order to interpret the metaphasic configurations found in *Luzula campestris* by way of following the course of meiosis in that species and its intraspecific hybrids. The schedule of Fig. 4, which illustrates these interpretations, ought, thus, in many points to be considered as a working hypothesis which has to be completed and verified by further investigations. This is especially true of the terminalisation of chiasmata and the separation of the chromatids. In a recent study of *Luzula purpurea*, KUSANAGI and TANAKA (1960) have given certain information which seems to support the assumptions made in connection with these phases. According to their observations the longitudinal, parallel separation of bivalents seems to start in the intermediate regions of the chromosomes, thus, supporting the assumption made in the present study. Other information they have given is that this separation as well as the longitudinal duality of each chromatid is evident already at late-pachytene stage, but that the bivalent chromosomes are usually held together in their distal regions. This statement indicates that the terminalisation of chiasmata and separation of chromatids might proceed almost simultaneously, and that the ends of the chromatids keep together until later stages, possibly until first metaphase. The ring-formation of chromatids at late diakinesis, revealed in the dumbbell-shape of the bivalents is, thus, a likely step in the development of the meiotic associations.

The formation of multivalents in material with diffuse centromeres seems to be rare in nature. The study of multivalents in *Luzula* has

mainly concerned artificially produced tetraploids or material containing translocations (DE CASTRO, CAMARA and MALHEIROS, 1949; DE CASTRO, NORONHA-WAGNER and CAMARA, 1954; LA COUR, 1953). However, the analysis of the formation of multivalents is mainly given in the study of the prophase stages. From later stages only exceptional bridge formations have been reported from material with X-ray induced translocations.

In the early investigations of *Carex*, which species is now supposed to have non-localized centromeres, certain natural polyploids with multivalent formation have been studied. TANAKA (1941) has studied the chromosome pairing of the tetraploid *C. siderosticta*, which has a very high frequency of multivalent formation. Many drawings from the different configurations are shown but they are all made in polar view. The multi-associations usually found are chains or rings; other configurations have been recorded in less than one per cent. The drawings of the ring and chain multivalents all show the type which could be expected if a multivalent formation analogous to the one in the schedule of Fig. 4 is assumed. In his cytological investigation of *Carex* species and hybrids, WAHL (1940) has given the same general pictures of the multivalent associations as TANAKA (1941) found in his material. Moreover, the former calls attention to the fact that each chromosome of the multivalents at first metaphase is always orientated in the metaphasic plane and that an equational division of the whole chromosome set, including the chains and the rings, is realized during the first meiotic division. Thus, at anaphase a set of chromosomes exactly duplicating the arrangement of the metaphase plate moves to each pole.

On summarizing the results of the present meiotic study, we shall endeavour to draw some parallels between the course of meiosis of the studied *Luzula campestris* and the one in materials possessing monocentric centromeres. As we have chromatid exchanges in *L. campestris*, we must genetically have a partially equational and partially reductional first meiotic division, just as we have in material with monocentric centromeres. Nevertheless, there is a fundamental difference between the two types of meiosis, cytologically revealed in the morphologically equational first meiotic division of material with non-localized centromeres. These conditions are made possible by a special type of meiosis, the course of which we do not as yet wholly understand. One of the fundamental differences between the two types of meiosis seems, however, to be the fact that in *Luzula* the chromatids start the separation at a very early stage, this separation being already completed at

the end of diakinesis, even if the associations are kept together at the terminal points of their chromatids. After separation the chromatids seems to act as the meiotic chromosomes of material with mono-centric centromeres. They orientate themselves around the metaphasic plane with their connecting points, i.e. their ends, fixed in the plane; they are thus arranged for the final separation of "free chromatids" at anaphase. As the chromatids are orientated around the metaphasic plane it appears as though each chromosome of an association would be orientated in that plane. This mode of orientation during the first meiotic division results in the characteristic ring and chain structures of the metaphasic multi-associations described in the studied material of *Luzula*.

Finally, we might add some remarks concerning the polyploid chromosome numbers occurring in material with non-localized centromeres (see p. 205). As mentioned above, it has been assumed that these polyploid chromosome numbers must have arisen in different ways. Firstly, we have the true polyploids of ordinary type formed by doubling or multiplication of the basic chromosome sets. Secondly, we have the variation of chromosome patterns designed as endonuclear polyploidy. The present cytological investigation of the hybrids between the true diploid with large AL-chromosomes and the endonuclear polyploids with small BL- or CL-chromosomes has made it possible to elucidate the origin of the endonuclear polyploids in the complex of *L. campestris*. As two BL- or four CL-chromosomes of the endonuclear polyploids have always been found to correspond to and to pair with one large AL-chromosome of the basic diploid set, the most likely origin of these BL- and CL-chromosomes seems to be that of a successive fragmentation of the AL-chromosomes. A theory of fusion of the small chromosomes into larger ones will, of course, give just as good an explanation of the pairing behaviour of the chromosomes. But as the diploids with the basic chromosome sets have the most common chromosome pattern of the complex and as the high-numbered, small-sized endonuclear polyploids are very rare, a fusion theory seems to be much less plausible than the fragmentation theory. In what way such a fragmentation can have taken place is, however, difficult to ascertain; we only know that each AL-chromosome must have given rise to two BL- or, probably secondarily, to four CL-chromosomes. In that way the endonuclear polyploids give rise to a sequence of chromosome numbers forming a geometric series, in contrast to the arithmetical ones found among the ordinary or true polyploids. Moreover, by such a

fragmentation system the total "chromosome length" of the endonuclear polyploids ought to be the same as the one of the basic, true diploids in spite of changes in chromosome numbers, whereas the nuclear content of the true polyploids is multiplied in the same degree as the chromosome numbers of the series. These consequences will explain the fact that the endonuclear polyploids in crosses always behave as diploids, i.e. they cross with the true diploids but never with the true, ordinary polyploids.

SUMMARY

(1) The chromosome patterns of the three studied hybrids of the genus *Luzula* are as follows: a) 2n=14 (10 AL+4 BL) originating from the cross 2n=12 (12 AL)×2n=16 (8 AL+8 BL); b) 2n=18 (6 AL+ +12 BL) originating from the cross 2n=12 (12 AL)×2n=24 (24 BL); and c) 2n=30 (6 AL+24 CL) from the cross 2n=12 (12 AL)×2n=48 (48 CL). (In morphological size one AL-chromosome corresponds to 2 BL- or 4 CL-chromosomes.)

(2) The hybrid 2n=10 AL+4 BL forms at first metaphase usually six chromosome bodies which have been interpreted as a formation of 4 homomorphic AL-bivalents and two heteromorphic associations of one AL- and two BL-chromosomes. The distribution of AL- and BL-chromosomes between the four cells of the pollen tetrads are demonstrated in Table 2. The hybrid 2n=6 AL+12 BL forms at first metaphase six heteromorphic associations of one AL- and two BL-chromosomes. The distributions of AL- and BL-chromosomes between the cells of the tetrads are recorded in Table 4. From the types of distribution of the AL- and BL-chromosomes it has been calculated that the first meiotic division must be cytologically equational and that the second one is reductional. From the kind of distribution of AL- and BL-chromosomes between the cells of the tetrads it has, moreover, been concluded that the presumed type of pairing at meiosis between one AL- and two BL-chromosomes is the most likely one to give the recorded distributions of chromosomes.

(3) The hybrid 2n=6 AL+24 CL forms at first metaphase 6 multi-associations together with numerous small CL-univalents. The multi-associations originate from the heteromorphic bivalents with one AL and 4 CL chromosomes as pairing partners. If chromatid exchanges occur between AL- and CL-chromatids during meiosis, a new type of intermediate-sized chromosomes may turn up in the mitosis of the

pollen tetrads (see Fig. 3). The distribution of AL-, intermediate-sized, and CL-chromosomes between the four cells of the pollen tetrads is recorded in Table 6. From the occurrence of intermediate-sized chromosomes in the pollen tetrads it is concluded that pairing and chromatid exchanges must occur between the AL- and CL-chromosomes during meiosis.

(4) The course of meiosis in *Luzula campestris* is described and a schedule (Fig. 4) has been made to explain the multivalent configurations found at first metaphase. The most fundamental difference between meiosis of *Luzula* and that of material with mono-centric centromeres seems to be the fact that in *Luzula* the chromatids separate at a very early stage and are held together only at their most terminal points. After separation the chromatids seem to act as the meiotic chromosomes of material with mono-centric centromeres, having their connecting points, i.e. their ends, fixed in the metaphasic plane and separating as "free chromatids" at first anaphase. During second metaphase the chromatids are again paired according to homology and separate in the same way as during first anaphase.

(5) From the pairing behaviour during meiosis between the large AL- and the small BL- or CL-chromosomes it has been concluded that the endonuclear polyploids cannot have arisen by a plain multiplication of the basic chromosome sets as in the case of the ordinary or true polyploids. The most likely origin of the endonuclear polyploids seems to lie in the fragmentation of the large AL-chromosomes of the basic diploid sets.

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TWO NEW CASES OF D_1 TRISOMY IN MAN

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TWO cases of the D_1 trisomy syndrome have been reported to date (PATAU, SMITH, THERMAN, INHORN and WAGNER, 1960; THERMAN, PATAU, SMITH and DEMARS, 1961). In each case there were seven rather than six chromosomes of the D group (classification by PATAU, 1960) which contains the chromosomes 13—15 (PATAU's as well as Denver system). Contrary claims notwithstanding, it is at present not possible to distinguish reliably between these chromosomes (THERMAN *et al.*, 1961). Nonetheless, it appears certain that in both cases the same chromosome was present in triplicate, the clinical resemblance of the two patients being by far too great to admit of any other interpretation. THERMAN *et al.* (1961) named the responsible chromosome D_1, proposing that in general a suffix to the group symbol be used to denote a chromosome that is identified by the phenotype of a trisomic rather than cytologically.

The present communication brings the number of D_1 trisomics we have investigated up to four. A detailed description, including autopsy findings, of the most recent cases (46 and 163) and additional information on the previous ones are being prepared for publication elsewhere. In the meantime, D_1 trisomics will no doubt be discovered at other places. Insofar as the identification of the extra chromosome cannot rest solely upon cytological evidence, the clinical picture having to be taken into account as well, it has seemed advisable to present promptly a brief characterization of the syndrome as it appears now, together with the cytological evidence.

Both patients died soon after birth. Therefore, only one specimen for cytological study could be collected from case 46: it was bone marrow

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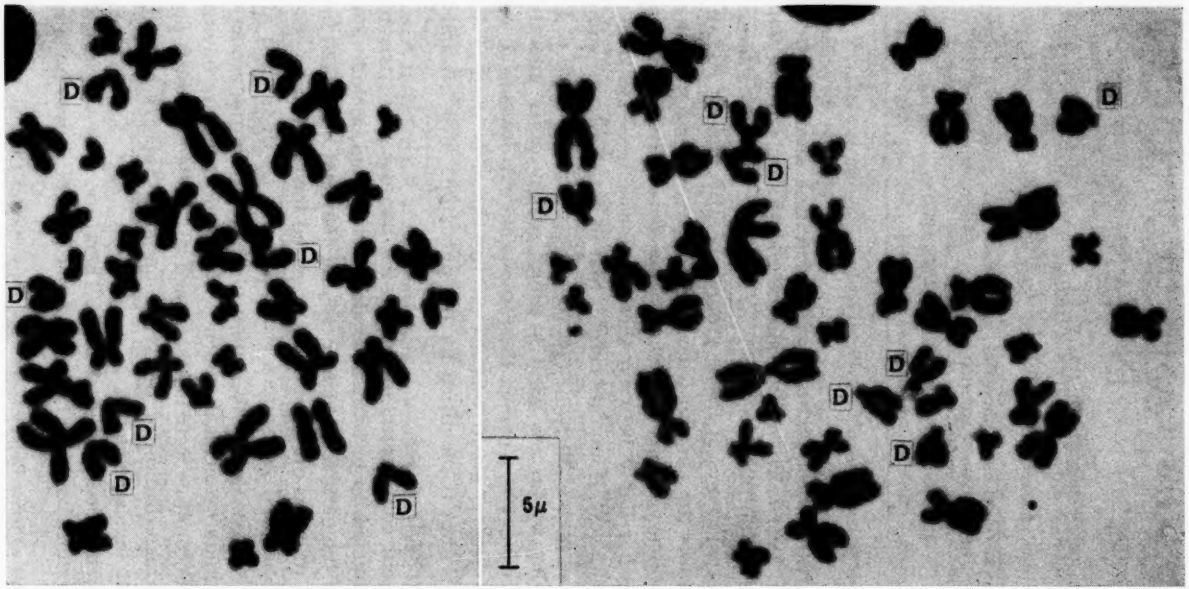

Fig. 1. The 47 chromosomes, including seven of the D group, of two D_1 trisomics. Feulgen, orcein. — Left: case 46, male (bone marrow). Right: case 163, female (blood culture).

aspirated after colcemide injection (for the techniques used see PATAU, THERMAN, SMITH and DEMARS, 1961). In case 163 short-term cultures of leukocytes were obtained from two blood samples (NOWELL's method as used by PATAU, THERMAN, SMITH, INHORN and PICKEN, 1961). All mitoses counted were analyzed as fully as possible. In both cases, there were invariably seven D chromosomes, otherwise the complement in all groups appeared entirely normal for the given sex (Fig. 1). The chromosome number was 47 (Table 1).

TABLE 1. *Chromosome counts, near-diploid cells only.*

Patient	Source	≤45	46	47	≥48	Total
Case 46	Bone marrow	—	1	19	—	20
Case 163	Blood culture	—	—	6	—	6
	,, ,,	—	1	14	—	15
	Total	—	2	39	—	41

The two patients were, as far as could be ascertained, unrelated to each other and to the earlier cases and there was nothing in either family history to suggest that the chromosomal abnormality was anything but trisomy resulting from accidental non-disjunction. There is no question that the pattern of congenital anomalies displayed by the four patients (Table 2) represents a well defined syndrome even though the clinical picture proved to be somewhat more variable than the first two cases had led us to expect. It will be noted that Table 2 does not list retroflexibility of the thumbs (formerly called "trigger thumbs") as

TABLE 2. *The D_1 trisomy syndrome.*

List of anomalies found in at least three of the four investigated infants (1 male, 3 females).

Anomaly	Frequency
Apparent mental retardation	4/4
Seizures	4/4
Apparent deafness	4/4
Eye defect (from severe microphthalmia to colobomata)	4/4
Hyperconvex finger nails	4/4
Cleft palate	4/4
Hare lip	3/4
Hemangiomata	3/4
Horizontal palmar creases	3/4
Interventricular septal defect	3/4

this was not found in the present patients, who had a flexion abnormality of the fingers instead. On the other hand, hyperconvexity of the nails had been noted before but not considered as particularly significant. Table 2 shows that it must now rank as a diagnostically valuable sign.

The concordance of the four cases can but mean that it was always the same chromosome, D_1, that was present in triplicate. Of course, in no single case can the possibility be ruled out that the extra chromosome was a translocation chromosome of which a major part had come from D_1. However, the probability of a translocation chromosome simulating morphologically the chromosome of which it contains a major part is certainly not great and it appears extremely unlikely that such a case should have happened more than once, if at all, in a sample of four patients. Table 2 may, therefore, be considered as sufficiently representative of the more common phenotypical consequences of D_1 trisomy to serve, together with the cytological findings, as basis for a reliable identification of at least most D_1 trisomics. Any case of apparent D_1 trisomy that deviates clinically in a striking manner from Table 2 should at the present time be regarded as ambiguous. It might represent an extreme variant of the syndrome but it might also be due to the extra chromosome being a translocation chromosome. For this as well as other reasons, it is of considerable importance to the cytogeneticist that the range of manifestation of the D_1 syndrome be determined as accurately as possible. It is hoped that authors who describe new cases of D_1 trisomy will not only state which of the anomalies listed in Table 2 are present but also, specifically, which are absent.

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VIABLE MUTANTS INDUCED IN BARLEY BY IONIZING RADIATIONS AND CHEMICAL MUTAGENS

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I. INTRODUCTION

IN cooperation with other workers, the authors have tried in a series of papers to elucidate the influence of chemical mutagens on a number of plant species, especially barley and peas. A comparison with the results obtained by ionizing radiations was always carried out.

The effects generally studied involve the following cytogenetic aspects:

lethality of the treated seed materials at seedling and plant stage;
chromosome disturbances (fragments and bridges in treated germinating seeds);
plant and spike fertility of randomly selected X_1 plants;
X_1-meiosis;
rates and types of X_2 mutations under greenhouse conditions (analysis at the seedling stage: especially chlorophyll mutations);
rates and types of X_2 mutations under field conditions ("field mutations");
continued field tests of viable mutations, chromosome translocations and chlorophyll mutations in subsequent generations.

Certain restrictions with regard to space and material are necessary for the completion of the yearly routine analysis. Nevertheless the X_2 and X_3 tests have increased considerably in the last few years, especially owing to the high mutation rates obtained with such chemicals as ethyleneimine (EI) and ethyl methanesulfonate (EMS).

Field mutations are of special importance in gene analysis and plant breeding. They are classified into four main groups: (1) chlorophyll mutations, (2) other lethal mutations, especially dwarfs, (3) aberrations showing so-called "translocation sterility" (TS), (4) viable mutations

(VM), involving morphological and physiological characters, in the latter case especially with regard to earliness and stiffness of straw. Minor changes can usually not be considered in the X_2 generation but are detected in the subsequent generations by special screening and selection methods. This holds true also of mutations involving disease resistance.

The expression "translocation sterility" was originally used in order to denote the sterility due to meiotic disturbances induced by ionizing radiations, chiefly chromosome translocations. In normal years this special type of sterility, regularly analysed in the X_2 (and X_3) generation, amounts to approximately 10—20 per cent of the seed set. Only part of the X_2 progenies displaying such a sterility show translocations which are cytologically detectable. True "translocation sterility" is rarer after treatment with chemical mutagens than after ionizing radiations.

The conclusions drawn from previous experiments may be summarized in the following manner (*cf.* GUSTAFSSON and TEDIN, 1954; EHRENBERG *et al.*, 1956 a and b; BLIXT *et al.*, 1960; EHRENBERG, 1960 a, b; GUSTAFSSON, 1960):

Ionizing radiations induce gross chromosomal rearrangements of various kinds, as well as "gene" (factor) mutations, both lethal and viable ones. After high dosages the resulting X_1 sterility is profound.

Certain chemical compounds, like nebularine, cause gene mutations without concomitant chromosome breakage and rearrangements. No X_1 sterility is present.

Other chemical mutagens, for instance 8-ethoxycaffeine, induce chromosome breakage and rearrangements, including translocation, but do not markedly raise the rate of viable mutations. A pronounced X_1 sterility may occur.

Some chemicals, *e.g.*, myleran, induce chromosome breakage but do not cause a corresponding amount of rearrangements. In fact, myleran also decreases the rate of X-ray induced translocations, when the seeds are presoaked in myleran solutions before X-irradiation (MOUTSCHEN, 1960). This proposed effect of myleran was recently disputed by RIEGER and MICHAELIS (1960).

Numerous chemicals break the chromosomes in a manner strikingly different from that of the ionizing radiations. In contrast to the random distribution in the latter case chromosome breaks are concentrated in heterochromatic regions of species where heterochromatin can be detected, or in centromere and constriction regions in species where heterochromatin is not distinguishable (*cf.* WETTSTEIN *et al.*, 1959).

Chemicals, like epoxides, epimines, and alkanesulfonic esters often lead to mutation rates decidedly higher than those obtained after appropriate treatments with ionizing radiations. This high efficiency (EHRENBERG, 1960 b) holds true with regard to viable mutations as well. Some compounds preferably induce gene mutations; detectable chromosome rearrangements and changes are rare (*cf.* EHRENBERG, 1960 b; FAVRET, 1960 b; HEINER *et al.*, 1960). In spite of this, X_1 sterility may be profound at high dosages.

Mutation spectra can be altered in various ways by the application of different mutagens. This is valid not only with regard to the types of chlorophyll mutations but also with regard to the rates of "translocation sterility" and viable mutations. In some cases a locus specificity has been detected, for instance in the case of neutron *contra* X- and γ-ray treatments, or ionizing radiation *contra* chemical mutagens (HAGBERG *et al.*, 1958; EHRENBERG *et al.*, 1959; FAVRET, 1959, 1960 a, b).

The chief purpose of this paper is to present further data on the biologic action of chemical mutagens and ionizing radiations in barley. Special interest will be focused on the origin of viable mutations.

Methods. — With regard to the methods used, details will be published in another context. Throughout the experiments, resting seeds or seeds pre-soaked for 16 or 24 hours have been treated, in the case of chemicals with a surplus of amount of solution (10 times the weight of the seeds) and at 20° C if not otherwise stated.

II. X_1 LETHALITY AND STERILITY

The immediate seed germination in the field is an indication of the effect of a special irradiation or chemical treatment. An appropriate figure representative of a special treatment is obtained if the percentage of surviving plants at the time of harvest is compared with that observed for the control material. In the production of mutations fast neutrons and X-rays are about equally efficient, but the former type of radiation is the more effective by a factor of 20—40 (EHRENBERG and NYBOM, 1954). With regard to seed lethality the corresponding figure is about 20 (*loc. cit.*). In Table 1 data are presented for a series of results obtained in the X_1 materials of 1958 and 1959.

The sterility of a material is generally determined by counting the fertile and sterile florets of 20 random X_1 plants, examining up to 10 spikes per plant, and of 10 control plants. Both plant and spike fertility are registered. Averaged for any series these two characteristics coin-

TABLE 1. *X_1 effects of radiations and chemical mutagens in barley.*

Mutagenic agent	Type of seed treated	Kind of treatment	Dosage giving		
			50 % X_1 lethality	50 % X_1 fertility	75 % X_1 fertility
Neutrons					
1959	Dry seeds 15 % H_2O		540— 720 rad	240— 360 rad	120 rad
1958	Dry seeds 15 % H_2O		600— 660 „	300— 360 „	120—180 „
X-rays					
1959	Dry seeds 10 % H_2O		3,400— 7,000 „	(3,400—7,000 „)	3,400 „
	13.3 % „		>10,000 „	—	6,800 „
1958	15 % „		15,000—17,000 „	(17,000 „)	8,500 „
Glycidol					
1959	Dry seeds	5 hours	0.30 %	—	>0.30 %
	„ „	5 „	0.10—0.30 %	—	—
1958	„ „	5 „	0.25—0.50 %	>0.50 %	0.25—0.50 %
Epichlorohydrine					
1958	„ „	5 „	0.10 %	—	—
Ethyleneimine					
1959	„ „	4 „ , sown immediately	0.05—0.07 %	0.10 %	0.07—0.10 %
	„ „	4 „ , sown after 1 day	0.03—0.05 %	<0.05 %	0.03 %
	„ „	20 „ , +4° C	0.06—0.12 %	<0.12 %	0.06—0.12 %
	Soaked seeds	4 „ , sown immediately	0.07—0.10 %	>0.10 %	0.07—0.10 %
	„ „	4 „ , sown after 1 day	0.05—0.07 %	0.10 %	0.05—0.07 %
Ethyl methanesulfonate					
1959	Dry seeds	24 „	0.33—0.50 %	0.33 %	0.17—0.25 %
	„ „	72 „ , +4° C	0.33—0.50 %	0.25—0.33 %	0.17—0.25 %
	Soaked seeds	24 „	0.25—0.33 %	0.33—0.50 %	0.17—0.25 %
Propyl methanesulfonate 1959	Dry seeds	24 „	0.40—0.80 %	>0.80 %	0.40—0.80 %

cide closely. In the following, therefore, only the average spike fertilities are considered. In Table 1 two such figures are presented, *viz.* 50 per cent and 75 per cent X_1 fertility.

Here we shall not deal with the differences in lethality and fertility found when applying sparsely or densely ionizing radiations. They have been considered previously in several articles (EHRENBERG *et al.*, 1952; EHRENBERG and NYBOM, 1954; EHRENBERG, 1960 b). Nor do we need to emphasize the influence of water content on radiation sensitivity (EHRENBERG, 1955).

With regard to chemical mutagens, the mode of treatment considerably affects the degree of lethality and fertility, as well as the mutation rates. If the seeds are sown immediately after a treatment with ethyleneimine, for instance, lethality, sterility, and mutation rates will become decidedly lower than in the case when treated seeds are stored for 24 hours previous to sowing. The effect of ethyleneimine is also strongly dependent on temperature during treatment, a low temperature (+ 4° C) giving less damage of all kinds than room temperature (+22° C).

III. CHLOROPHYLL MUTATIONS IN GREENHOUSE TESTS

1. Maximum mutation rates

Detail data will not be presented in the following analysis. For each kind of mutagenic treatment (neutrons, X-rays, ethyleneimine, ethyl methanesulfonate) we have selected the six highest mutation rates, obtained in different series during the years 1958—1960.

Neutrons:	13.8*	10.0*	10.0	9.9	9.6	9.4*	%
No. of spike progenies	87	270	688	378	261	395	
X-rays:	(14.8*)	11.7*	11.2*	(11.1)	10.9*	9.9	%
No. of spike progenies	27	264	241	9	285	344	
EI:	28.2*	27.0	25.4	24.6	23.4*	23.4	%
No. of spike progenies	78	337	236	57	205	154	
EMS:	47.9	46.0	38.9	38.6	34.0	23.3	%
No. of spike progenies	76	113	126	83	47	172	

The maximum level of chlorophyll mutations generally amounts to 10—11 per cent for both neutrons and X-rays. In some series rates up to 13 or 14 per cent were found. In the instances denoted by asterisks the lateral tillers were cut off at anthesis, leaving the three primary tillers intact until harvesting. In the case of the opposite treatment, *i.e.*,

cutting off the primary tillers, only lateral tillers developing, lower mutation rates were generally obtained with all mutagenic agents tried (EHRENBERG and v. WETTSTEIN, unpubl.).

In the case of treatments with ethyleneimine the corresponding maximum lies around 27 per cent, *i.e.* twice the maximum rate obtained with ionizing radiations. The sulfonates are still more efficient. Ethyl methanesulfonate gives rates up to 50 per cent, as described previously by HESLOT *et al.* (1959), EHRENBERG (1960 a), and GUSTAFSSON (1960). Actually rates up to 60 per cent have been reached in recent experiments (X_1 and X_2: 1960).

Numerous other chemicals and radiations have been tried. Ethylene oxide, as well as some of its derivatives, are quite efficient, especially glycidol, which induces mutations at rates decidedly higher than those obtained when neutrons or X-rays are used. Diethyl sulfate, which according to HEINER *et al.* (1960) is highly mutagenic, gave rates in our experiments below the glycidol rates but above those of neutrons and X-rays. Various alkanesulfonates have been tried with considerable success, *e.g.* the propyl methanesulfonate and the ethyl ethanesulfonate. These data will be considered in a following paper, as will results concerning other kinds of radiation.

2. X_1 sterility and X_2 mutation rates

As mentioned previously, all highly efficient mutagenic agents, whether ionizing or chemical, induce a more or less pronounced X_1 sterility. This may be chromosomal, genic or physiological in character. In the case of ionizing radiations much of the sterility is chromosome conditioned, as is evident from an analysis of the X_2 and X_3 generations and the high rates of chromosome translocations. To what extent inversions, duplications, and deficiencies contribute to the X_1 sterility, is not definitely known. KAPLAN (1949) tried to correlate different degrees of sterility with different kinds of chromosomal aberrations, especially deficiencies, but his interpretations are conjectural. Applying ethyleneimine there is a considerable amount of chromosomal aberrations in the roots of seeds treated at high doses (J. and M. MOUTSCHEN, unpubl.). After a treatment with ethyl methanesulfonate chromosomal aberrations are scant or absent, at least up to doses which give very high mutation rates (FAVRET, 1960 b; NATARAJAN, unpubl.; *cf.* EHRENBERG, 1960 b; RIEGER and MICHAELIS, 1960). Whether there is also, especially in the case of these efficient mutagens, a physiological, non-hereditary sterility must be left undecided at present.

The contrast between ionizing radiations and these two mutagens becomes apparent when comparing the X_1 sterilities and the X_2 mutation rates. In Table 2 and Fig. 1 the average mutation rates of neutrons, X-rays, EI and EMS are arranged according to a successive decrease in fertility (increase of sterility). For neutrons there is no obvious "threshold value", *i.e.* no mutations appear without a noticeable decrease in X_1 fertility, possibly owing to the fact that the neutron-induced sterility rises more or less linearly with dosage, as do mutation rates. (It must be pointed out, however, that the occurrence of such a "threshold", regarded as a step, depends on the accuracy of the analysis.) The curve becomes distinctly flattened at the point of 50 per cent fertility.

With X-rays there is a definite "threshold value", *i.e.* mutations occur in the offspring of X_1 plants without any detectable decrease in fertility. This is in line with a proposed exponential rise of sterility. The "threshold value" lies around 1.7 per cent mutations. Generally, the curve ends at 40—50 per cent X_1 fertility. Almost complete seed and seedling lethality takes place at this stage of X-ray damage. Owing to the occurrence of a "threshold value", the first part of the X-ray curve is situated slightly above that of neutrons.

Ethyleneimine gives a picture like the one obtained after X-irradiation. The curve ends abruptly around 50 per cent sterility. There is a "threshold value" of 4 per cent. The curve steeply ascends to an average mutation rate of approximately 20 per cent at 55 per cent X_1 fertility. With ethyl methanesulfonate there is also a "threshold value", in the present material at approximately 3 per cent. The curve rises very steeply up to a mean mutation rate of almost 50 per cent at an X_1 fertility of 35 per cent.

From the data shown in Table 2 we can calculate the amount of sterility induced simultaneously with a rise of the mutation rates by 1 per cent. This can be done in different ways: (1) All the data of a mutagenic treatment are averaged, irrelative of any elimination and lethality at high sterilities or of the occurrence of threshold values, (2) like in (1) but correction is made on account of the occurrence of threshold values. (3) Only values above 50 per cent X_1 fertility are considered, the "threshold values" included, too, and (4) like in (3) but correction is made on account of the occurrence of threshold values.

(1) Every increase of mutation rate by 1 per cent follows after an X_1 sterility of 7.5 % in the case of neutrons, of 5.1 % in the case of X-rays, of 1.3 % in the case of ethyleneimine and of 1.1 % in the case of ethyl methanesulfonate.
(2) The X_1 sterility amounts to 7.5, 7.6, 2.3 and 1.3 per cent, respectively.
(3) „ „ „ „ „ 5.6, 4.6, 1.3 „ 0.9 „ „ „
(4) „ „ „ „ „ 5.6, 6.9, 2.3 „ 1.1 „ „ „

TABLE 2. *X_1 fertility and average X_2 mutation rates.*

(X_1: 1957—1959.) (Greenhouse mutations.)

	% X_1 fertility (control = 100 %)										
	— 100	— 90	— 80	— 70	— 60	— 50	— 40	— 30	— 20	— 10	— 0
Neutrons	—	(1.0 %)	3.2 %	4.4 %	6.0 %	7.9 %	6.1 %	8.4 %	7.6 %	(5.6 %)	—
No. of series	—	1	4	4	6	7	4	9	10	1	—
X-rays	1.7 %	3.2 %	4.7 %	4.3 %	7.0 %	7.1 %	—	(4.6 %)	—	(11.1 %)	—
No. of series	3	10	6	14	10	9	—	2	—	1	—
EI	4.1 %	6.2 %	12.6 %	16.4 %	13.5 %	17.7 %	—	—	—	—	—
No. of series	6	27	19	14	4	4	—	—	—	—	—
EMS	3.0 %	8.3 %	19.6 %	—	31.0 %	39.5 %	—	(47.9 %)	—	(34.0)	—
No. of series	2	7	3	—	2	3	—	1	—	1	—

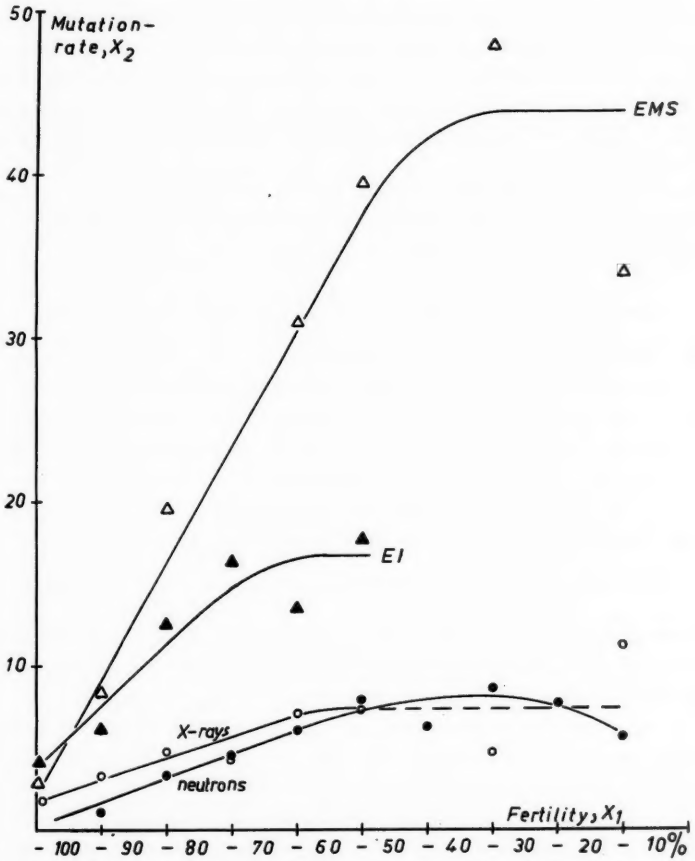

Fig. 1. X_1 fertility (in per cent of control) and rates of chlorophyll mutations (greenhouse data) using ionizing radiations (neutrons and X-rays) and chemical mutagens (ethyleneimine, EI, and ethyl methanesulfonate, EMS).

The high sterility in the case of ionizing radiations, induced for each per cent of chlorophyll mutations, explains the difficulty in reaching mutation rates above 10—14 per cent when neutrons or X-rays are applied. For neutrons this is simply not possible, since parallel to each per cent of chlorophyll mutations such a high amount of chromosome and other disturbances is produced, that with 14 per cent mutations full sterility will be reached. For X-rays and ethyleneimine the X_1 seed

lethality prevents a rise of mutation rates above 10 and 25 per cent, respectively. Chromosomal and other disturbances are less frequent for corresponding mutation rates when ethyleneimine is applied instead of X-irradiation. Ethyl methanesulfonate is most efficient of the four mutagens, since it induces only slight X_1 lethality and sterility even at high mutation rates.

The difference between ionizing radiations and chemical mutagens is indeed striking. Definitely higher threshold values seem to be obtained with EI and EMS than with X-rays.

3. Chlorophyll mutations and the spectrum of mutations

In previous experiments (EHRENBERG *et al.*, 1959) ethyleneimine caused a spectrum of chlorophyll mutations different from that obtained with neutrons or X-rays. Refraining from details, we may state that similar results were obtained also in subsequent years (1958/59 and 1959/60). The following compilation presents recent data, also those of the EMS-treatments. In the last two years the mother line consisted of the Svalöf malting barley 02102 instead of Svalöf's Bonus.

X_2 Year	Treatment	Mother line	Frequency of			Sum of mutants
			Albina	Viridis	Others	
1957/58	Neutrons	Bonus	42.1 %	41.0 %	16.9 %	183
„	X-rays	„	40.2	40.2	19.5	87
„	EI	„	24.0	51.8	24.2	421
1958/59	Neutrons	02102	34.9	46.9	18.2	527
„	X-rays	„	38.4	45.8	15.8	614
„	EI	„	27.4	52.3	20.3	1,003
1959/60	Neutrons	„	36.2	43.5	20.2	124
„	X-rays	„	36.4	43.9	19.6	239
„	EI	„	28.5	45.5	26.0	393
„	EMS	„	24.2	50.9	24.8	420

Comparing ionizing radiations and chemical mutagens, we find a definite contrast in behaviour: (1) albina mutants are relatively less common after treatments with chemical mutagens (*cf.* v. WETTSTEIN *et al.*, 1959, Fig. 7), (2) viridis mutants, as well as rare mutants ("others"), increase in number. The statistical analysis shows, with high significance, that the mutation spectra obtained after irradiation are different from those produced by EI and EMS. No clear average

difference is present either between neutrons and X-rays, or between EI and EMS, although the frequency of albina mutants is lower with EMS than with EI.

However, if the mutation rates of EI- and EMS-treatments are arranged according to the degree of X_1 fertility, there is also here some evidence of a difference in behaviour. At high X_1 fertility albinas are much more common with EI-treatments than with EMS. At decreasing fertility there is a controversial trend in behaviour, a decreasing rate of albinas with EI and an increasing rate with EMS. For the two groups of high X_1 fertility (80—90 per cent and above 90 per cent) the surplus of albinas in EI-treatments is significant ($P=0.03$ and 0.05, respectively). At lower fertility the difference is evened out and converted to a deficiency of albinas with EI and a corresponding surplus with EMS. Since this is the first spectrum comparison made between the two agents, we do not want to overemphasize the difference. If real, it may mean a different action of the mutagens on individual groups of chlorophyll genes, as suggested for other chemical mutagens by v. WETTSTEIN *et al.* (1959).

IV. FIELD MUTATIONS

1. General presentation

Table 3 gives a survey of field aberrations found in the X_2 generation. The data of 1958/59 were previously published. They are here treated in more detail.

The aberrations regularly considered in our routine analysis of field mutations are certainly not exhaustive. A largely unknown group consists of aberrations with "recessive sterility", which is often extreme in expression and segregates in further generations according to a 3:1 scheme. Actually, these aberrations are almost as frequent as chlorophyll mutations (GUSTAFSSON, 1947, p. 47). They have not been specially considered in the last years of experimentation. Neither is the group, called "dwarfs and lethals", fully analysed. In spite of this and other imperfections the routine analysis gives a rough idea of mutation rates and mutation spectra also in field materials.

Two details are evident from the table. First, absolutely and relatively seen, viable mutants obtained with chemicals are on an average much more numerous than those produced by ionizing radiations (columns 9 and 10, *cf.*, however, the foot-note of Table 3). Secondly, this is connected with the fact that progenies showing partial sterility ("translocation sterility", TS) are less represented in the case of chemicals than in the case of ionizing radiations, especially neutrons (column 7 and Figs. 2 and 3). On the other hand, chlorophyll mutations, dwarfs and lethals show approximately the same average proportion to viables

TABLE 3. *Field aberrations.*

X_2: 1958, 1959, 1960 (X_1: 1957, 1958, 1959).

Year / Mutagenic agent	Number of X_2 spike progenies	Field progenies with					Proportion		Per cent mutations		
		Chlorophyll mutations	Dwarfs and lethals	So-called translocation sterility	Viable mutants	Sum	$\frac{1+2}{4}$	$\frac{3}{4}$	Total (1--4)	Viables (4)	Viables of total (4/1—4)
		1	2	3	4	5	6	7	8[1]	9[1]	10
1958											
Neutrons	1,634	106	30	408	34	578	4.3	12.0	35.4	2.1	5.9
X-rays	1,348	72	12	241	41	366	2.0	5.9	27.1	3.0	11.2
Ethylene oxide	2,557	117	15	174	55	361	2.4	3.2	14.1	2.2	15.2
Ethyleneimine	1,497	276	68	409	141	894	2.4	2.9	59.7	9.4	15.8
1959											
Neutrons	1,010	89	35	461	46	631	2.7	10.0	62.4	4.6	7.3
X-rays	1,441	149	37	544	50	780	3.7	10.9	54.1	3.5	6.4
Diethyl sulfate	311	45	10	72	12	139	4.6	6.0	44.7	3.9	8.6
Glycidol	397	48	17	94	18	177	3.6	5.2	44.6	4.5	10.2
Epichlorohydrine	239	19	2	22	7	50	3.0	3.1	20.9	2.9	14.0
Ethyleneimine	3,662	442	110	927	177	1,656	3.1	5.2	45.2	4.8	10.7
1960											
Neutrons	1,130	64	21	270	42	397	2.0	6.4	35.1	3.7	10.6
X-rays	1,676	93	30	199	42	364	2.9	4.7	21.7	2.5	11.5
Glycidol	447	86	18	132	30	266	3.5	4.4	59.5	6.7	11.2
Ethyleneimine	2,022	272	87	566	136	1,061	2.6	4.2	52.5	6.7	12.8
Ethyl methanesulfonate	1,992	489	152	675	222	1,538	2.9	3.0	77.2	11.2	14.4
Propyl methanesulfonate	192	23	6	33	15	77	1.9	2.2	40.1	7.8	19.5

[1] The mutation rates given in columns 8 and 9 are the averages of all series tested in the respective years. Therefore the values cannot be used to illustrate the efficiency of the agents at optimum doses and other conditions.

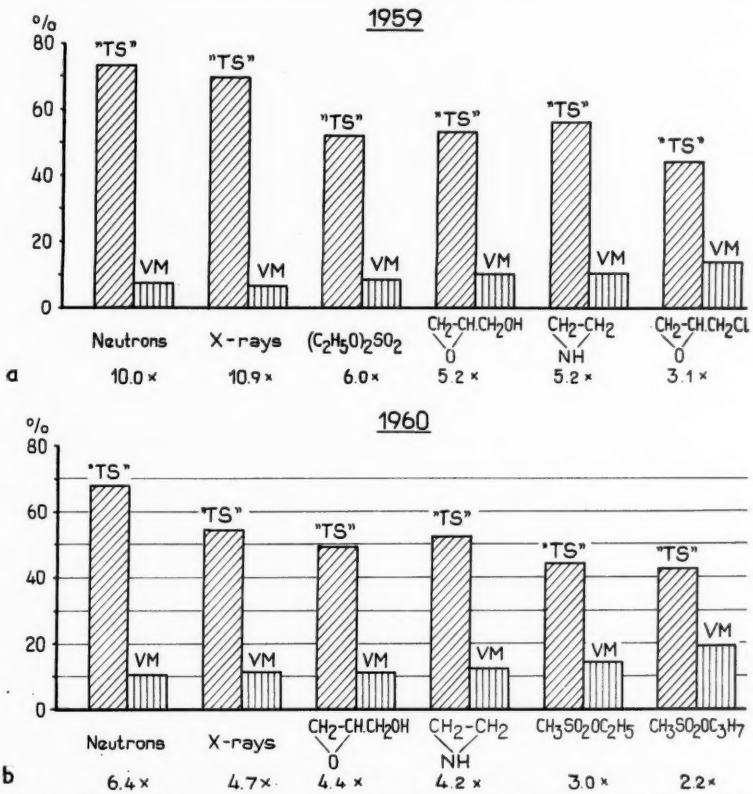

Fig. 2. The mutual proportions of X_2 progenies containing "translocation sterility" ("TS") and viable mutants (VM) (field data of a: 1959 and b: 1960).

throughout the list of mutagens tested (*cf.* p. 261). Partial sterility, induced by neutrons and X-rays, is to a high extent conditioned by chromosome irregularities in the X_2 meiosis, commonly translocations. In the case of EI-treatments, and even more so when EMS is used, this partial sterility rather depends on gene mutations (or deficiencies) and to a minor degree on gross chromosome irregularities. According to rough calculations, one half or two thirds of the X-ray progenies showing partial sterility of the TS-type should be due to chromosome translocations, the rest to other causes. If neutrons are applied, there is a higher proportion of partial sterility caused by chromosomal rearrangements than in the case of X-irradiation.

cannot be used to illustrate the efficiency of the agents at optimum doses and other conditions.

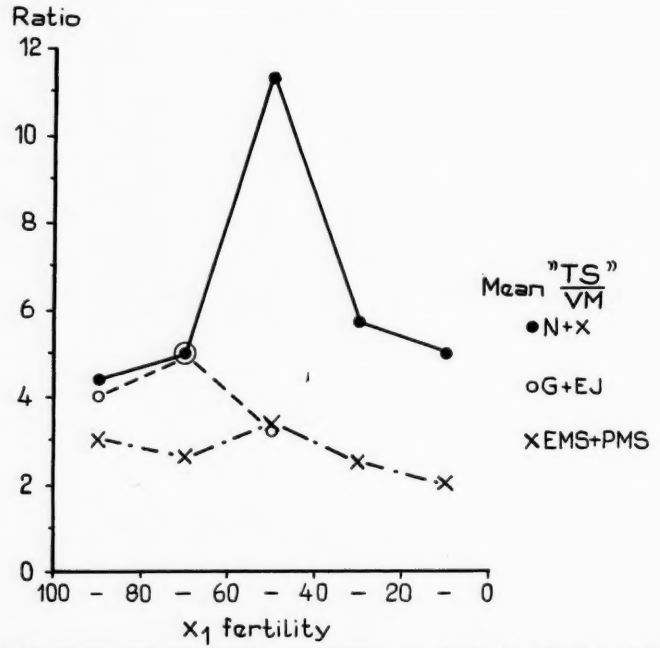

Fig. 3. The mutual proportions of X_2 progenies containing "translocation sterility" and viable mutants in relation to X_1 fertility. (N=neutrons, X=X-rays, G and EI= glycidol and ethyleneimine, EMS and PMS=ethyl and propyl methanesulfonate.)

The table further indicates the great possibilities involved in mutagenic treatments. The rates of induced field aberrations calculated as averages of all materials amount to 50—60—70 per cent of the spike progenies tested. By appropriate treatments and by special selection of the X_1 material, the aberration rates will reach considerably higher values. This is so even with regard to the viable mutations, which in the series presented here occur at a rate up to approximately 10 per cent of the X_2 progenies when chemical mutagens are used.

2. X_1 sterility and field aberrations

Subdividing the X_2 aberrations of Table 3, according to the X_1 fertility of the treated series, we obtain the situation given in Table 4, which presents field data of 1960.

This compilation is of interest from several points of view. It indicates that the maximum amount of field aberrations both in the case

TABLE 4. *X_1 fertility and total rates of the field aberrations studied* (X_2: 1960).

Mutagenic agent	Per cent X_1 fertility (control = 100 %)											
	> 100	–	80	–	60	–	40	–	20	–	0	
	%		%		%		%		%			
Neutrons	14.3	(67)	29.0	(69)	49.0	(79)	69.2	(182)	—			
X-rays	15.8	(117)	25.4	(196)	29.2	(40)	—		[37.9	(11)]		
Glycidol	49.6	(129)	73.3	(137)	—		—		—			
Ethyleneimine	42.4	(626)	77.2	(288)	85.5	(147)	—		—			
Ethyl methanesulfonate	44.0	(577)	117.1	(254)	148.7	(528)	185.7	(169)	[111.1	(10)]		
Propyl methanesulfonate	[14.8	(21)]	112.0	(56)	—		—		—			

Within brackets: number of aberrant X_2 progenies.

of ionizing radiations and chemical mutagens is found at fairly low fertility values (20—40—60 %). At all fertility values the chemical mutagens are superior in efficiency, giving aberration rates of up to 85 per cent with EI, 110 per cent with PMS and 185 per cent with EMS, *i.e.* values three times higher or more than those obtained with ionizing radiations. The high rates of chemically induced aberrations are noteworthy at complete or almost complete fertility (80—100 per cent), where the chemicals show rates close to 50 per cent. This is in agreement with the occurrence of "threshold values" (p. 249), *i.e.* mutations arise without concomitant X_1 sterility.

The high aberration rates appearing in chemical treatments are illustrated by the fact that there arose in the range of 40—60 per cent X_1 fertility:

from 172 X_1 spikes and 57 X_1 plants (ethyleneimine) 147 aberrants, *i.e.* average rates of 86 and 258 per cent, respectively;

from 355 X_1 spikes and 105 X_1 plants (ethyl methanesulfonate) no less than 528 aberrants, *i.e.* 149 and 503 per cent, respectively.

Moreover, these aberration rates are minimum frequencies. We can safely expect at least twice or thrice as high total aberration rates, if analysing the induced variation completely and including also mutations with minor effects. This implies rates higher than 1,000 or 2,000 per cent per plant progeny, *i.e.* more than 10 or 20 different aberrations may arise per X_1 plant. The repeated treatment of seed materials with EI or EMS (alone or in combination with ionizing radiations) will in a couple of years produce hundreds of aberrations per plant offspring, all of which may be built up on one single plant of the highest productive trade variety used in an area. Hybridization *breaks down* the harmonious genotypes obtained in the continuous plant breeding. Mutation leaves them in principle *intact*, except for the modifying or drastic single-step mutations or, in the case of chromosomal rearrangements, the altered gene interactions and balances.

In the case of a stagnant variation in crop plants of any kind, such a repeated treatment with chemical mutagens and ionizing radiations will enrich the variation considerably by producing new genes and karyotypes.

V. VIABLE MUTANTS

1. Mutation rates

The central matter in this mutation analysis concerns the viable mutations, many of which, whether they are morphological or physiological in character, drastic or modifying, have potential value in plant breeding. The highest rates obtained with various mutagens, as well as the corresponding kinds of treatment, are visualized in the following compilation, comprising figures based on a representative number of progenies (*cf.* EHRENBERG *et al.*, 1959, p. 361):

Neutrons	— 1960:	9.3 %,	360 rad	
	1959:	8.3 %,	180 „	
	1958:	3.6 %,	240 „	
X-rays	— 1960:	4.5 %,	10,200 „	
	1959:	4.9 %,	14,900 „	
	1958:	5.4 %,	6,800 „	
Glycidol	— 1960:	9.1 %,	0.3 %	solution, resting seed, 5 hours of treatment
	1959:	4.5 %,	0.25 %	solution, resting seed, 5 hours of treatment
Ethyleneimine	— 1960:	16.9 %,	0.12 %	solution, resting seed, 20 hours of treatment, low temperature (+4° C)
	1959:	9.8 %,	0.03 %	solution, soaked seed, 6 hours of treatment
	1958:	18.4 %,	0.2 %	solution, soaked seed, 2 hours of treatment
Ethyl methanesulfonate	— 1960:	28.6 %,	0.33 %	solution, resting seed, 3 days of treatment, low temperature (+4° C)
		24.4 %,	0.25 %	solution, soaked seed, 1 day of treatment

It was shown in the paper cited that the rates of "translocation sterility" in the X_2 generation did not, even in the case of maximum mutation rates, reach higher values after treatments with chemical mutagens than those obtained after irradiation. In the glycidol and ethyleneimine materials of 1959 and 1960 there is a similar condition. In the most efficient treatments with EMS, however, higher rates of "translocation sterility" were recorded than in any radiation experiment (71 % "translocation sterility" in the case of EMS in contrast to 40 % for neutrons and 44 % for X-rays).

The rates of viable mutations in relation to X_1 fertility are given in Table 5. It is evident, here too, that the maximum frequency of viables appears in the offspring of sterile X_1 plants. Their frequency is approximately the same whether neutrons or X-rays are used. Glycidol and

TABLE 5. *X_1 fertility and rates of viable mutants (X_2: 1960).*

Mutagenic agent	Per cent X_1 fertility (control = 100 %)												Sum of viables
	> 100	—	80	—	60	—	40	—	20	—	0		
	%		%		%		%		%				
Neutrons	1.9	(9)	2.9	(7)	3.2	(5)	8.0	(21)	—				42
X-rays	2.2	(16)	2.9	(22)	2.2	(3)	—		[3.4	(1)]			42
Glycidol	5.0	(13)	9.1	(17)	—		—		—				30
Ethyleneimine	5.6	(83)	8.3	(31)	12.8	(22)	—		—				136
Ethyl methanesulfonate	6.3	(82)	18.9	(43)	19.4	(69)	28.6	(26)	[22.2	(2)]			222
Propyl methanesulfonate	4.2	(6)	18.0	(9)	—		—		—				15

Within brackets: number of different viable mutants isolated.

ethyleneimine occupy a position between ionizing radiations and ethyl methanesulfonate. In the case of EMS-treatments the rates of viable mutants reach mean values of 20—30 per cent. In the region of 60—80 per cent X_1 fertility, EMS-viables are six times as frequent as the viables induced by neutrons or X-rays. Even in the high fertility group (80—100 % fertility), EMS viables are three times as common as in the case of ionizing radiations.

Viables occur in approximately equal proportion to other field aberrations throughout the X_1 fertility range when chemical mutagens are used. After irradiation the ratio of translocation sterility, lethals and dwarfs to viables is higher in the offspring of sterile X_1 plants. Owing to the great increase of chromosome rearrangements with rising radiation dose and sterility, aberrations like "TS", lethals and dwarfs will prevail at high X_1 sterilities. If chemical mutagens are used, however, the ratios of viables will be the same throughout the fertility range.

The figures in Tables 4 and 5 indicate that by appropriate selection techniques we may accumulate viables, which are free from other aberrations to the same extent, whether we extract them from 20—60 per cent fertile X_1 plants (EI and EMS), with very high rates of viables, or from 90—100 per cent fertile X_1 plants (N+X) with low rates of viables.

The relationships with regard to viables, on one hand, and lethals and sterilities on the other, are visualized graphically in Fig. 4. The neutron data are put as base reference=1 in all X_1 fertility ranges. Without exploiting the material too heavily, the following conclusions seem justified:

Generally X-rays and neutrons behave in like manner with regard to the distribution of viables and other field aberrations. However, the rates of viables and other aberrations appear slightly higher in low fertility ranges if neutrons are used instead of X-rays.

With ethyleneimine at almost full X_1 fertility, the rates both of viables and other aberrations increase in a similar manner. At low fertilities, on the contrary, there is a distinct surplus of EI-viables, relative to other aberrations.

If EMS is used there is a conspicuous gap between the rates of viables and other aberrations, although they approach one another at high and low fertilities.

Finally, the rate of "other aberrations" induced in highly fertile X_1 plants by means of PMS lies at the level of the corresponding neutron rate. The rate of viables is distinctly higher. Both types of aberrations

increase in frequency at a lower fertility, but the viables still more so than other aberrations.

The graphs reflect the conditions previously related, *viz.* that using ionizing radiations "translocation sterility" and lethals of various kinds successively increase relative to viables in low fertility ranges. The data also indicate that the mutation spectrum changes with the type of mutagen.

The differences no doubt are connected with the occurrence or absence of gross chromosomal alterations. Many lethal mutations, not only partial sterilities, are associated with chromosome rearrangements, especially in the case of ionizing radiations. Indeed, this may be the case with viable mutations, too, as suggested by FAVRET's studies (1960 a) on mildew resistance in barley. However, when chromosomal aberrations are few, like in EI- or even more so in EMS-treatments, viable mutations generally become more abundant, relatively seen, and most of them behave like gene mutations.

Viable mutations may appear also when there is no evident X_1 sterility present. Such "threshold values" occur in the case of X-rays, EI- and EMS-treatments. At decreasing X_1 fertility there is a rise in mutation rates rather similar to that found in the case of greenhouse mutations (Fig. 1). The average sterility induced parallelly with an increase of the rate of viables by 1 per cent is (calculation method 1, p. 249),

for neutrons	10.1	per	cent
„ X-rays	7.8	„	„
„ EI	3.0	„	„
„ EMS	2.1	„	„

2. Types of viable X_2 mutants

To be as complete as possible the extraction of viable mutations should be carried out in the X_3 (or X_4) generation and should comprise progenies of every X_2 plant. Otherwise, only part of the induced total variation is realized. This is specially the case when the mutation rates are high or a certain mutagenic agent is repeatedly applied. The data just reported indicate that mutation rates of several hundred per cent (calculated per spike progeny) or several thousand per cent (calculated per plant progeny) can be reached. A full analysis of mutant types indeed becomes necessary when the refinement of mutagenic treatments now possible is fully utilized. Different mutagens may be combined, for instance ionizing radiations *per se*, chemical agents *per se*,

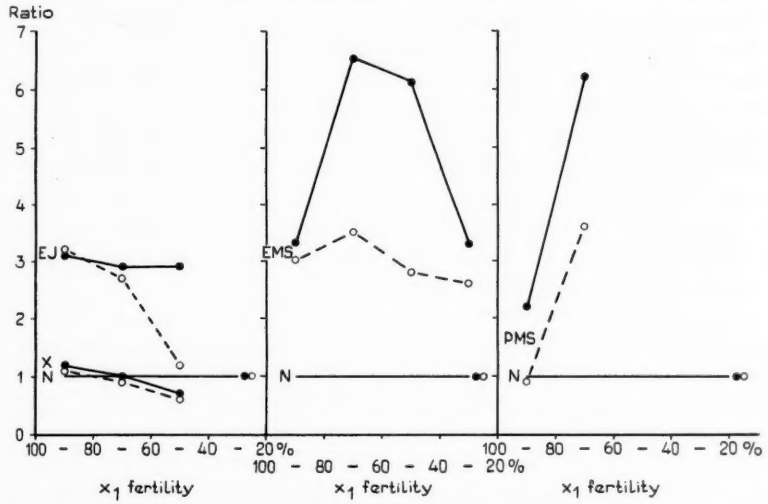

Fig. 4. X_1 fertility and relative rates of X_2 progenies containing viable mutants (●), as well as "translocation sterility" and lethal mutants (○). Rates of X-rays, ethyleneimine, ethyl and propyl methanesulfonate treatments expressed in proportion to the corresponding neutron data as base references (abbreviations as in Fig. 3).

or a mixture of two or several types of mutagens, applied once or repeatedly in two or more subsequent generations.

The present model analysis did not aim at a complete break-up of the total variation achieved by different mutagens. We preferred to isolate special kinds of mutations in the second generation and then to test them in the third and fourth generations. In our routine analysis we distinguished between the following groups of mutant types:

erectoides mutants;
bright-green „ ;
short-awned „ ;
six-row „ ;
mutants with irregular spikes;
„ „ lemma-like glumes ("macrolepis", NÖTZEL, 1952);
early mutants;
lax-eared „ ;
stiff, erect mutants; and
"other" mutants.

The group of "other" mutants comprise rare morphological changes, often drastic ones, as well as less conspicuous or small changes, attracting the trained eye already in the X_2 generation. All variants recognized are further studied in the third generation. In general, those not confirmed are discarded. A more complete segregation of the induced variation was accomplished by GUSTAFSSON (1941, pp. 240—241; 1942, p. 63; 1951, p. 277; *cf.* also GUSTAFSSON and v. WETTSTEIN, 1956/57, p. 685) in restricted materials of X-ray treatments. The studies indicated a "total" mutation rate of approximately 100 per cent per plant progeny for 10,000 r-units, dry dormant seed being irradiated.

The *erectoides* mutations may be briefly discussed here. In this case it was proven that the mutants are caused by changes in a great many loci (HAGBERG, 1960). Disregarding locus property, they were calculated to arise at a rate of one per 25 chlorophyll mutations (GUSTAFSSON and MAC KEY, 1948). The mutation rate was approximately 0.18×10^{-6} per spike progeny and r-unit. With regard to the parallel occurrence of chlorophyll mutations, identified under field conditions, the following data may be mentioned. In 1960 there were 89 cases of erectoides mutations. Simultaneously, 1,027 chlorophyll mutations were recognized, a proportion of 1:11.5. In 1959 the corresponding figures were 42 erectoides mutations and 673 chlorophyll mutations, a proportion of 1:16.0. These figures must be considered with a good deal of reservation, since the rates vary between different years, as well as between different mutagenic treatments.

In fact, the wide variation in mutation rates is illustrated by the figures calculated per spike progeny and r-unit, for the years 1955—1960:

$1955 — 3.0\times10^{-6}$	"Svalöf's Bonus"
$1956 — 0.4\times10^{-6}$	„ „
$1957 — 0.8\times10^{-6}$	„ „
$1958 — 1.9\times10^{-6}$	„ „
$1959 — 0.1\times10^{-6}$	"Svalöf's 02102"
$1960 — 1.2\times10^{-6}$	„ „

This variation in mutation rate partially depends on the climatic conditions prevailing, since ear density (the chief selection property of the erectoides mutants) varies in expression from year to year. It also depends on the locus mutated as well as the allele arisen.

There is a distinct influence on mutation rate exerted by a change in water content in the X_2 material of 1960. The irradiated seed mate-

rial was equilibrated prior to irradiation according to EHRENBERG's method (1955). The rates are expressed in number of erectoides mutations per spike progeny.

Dose	Water content						Sum
	7.6 %	*10.1 %*	*11.3 %*	*13.3 %*	*15.5 %*	*20.4 %*	
1,000 r	0/65						0/65
2,000 r	1/75	1/59	0/82				2/216
4,000 r	1/150	2/152	0/64	0/73	0/77	0/68	3/584
8,000 r		(0/29)	0/165	0/79	0/73	0/59	0/405
12,000 r				2/132	1/140	0/137	3/409
Sum	2/290	3/240	0/311	2/284	1/290	0/264	8/1679
	5/530		2/595		1/554		
Per cent	0.94		0.34		0.18		
Rate per r-unit and spike progeny	3.4×10^{-6}		0.2×10^{-6}		0.1×10^{-6}		

Consequently, the dry seed material (7—10 %) gives higher erectoides rates than normal seeds (11—13 %) and these in their turn higher rates than the moist seeds (15—20 %).

A similar type of metabolic influence was discussed in previous papers dealing with the alboxantha and tigrina mutations of barley (D'AMATO and GUSTAFSSON, 1948, p. 189). The alboxantha mutations concentrated to the offspring of moist or presoaked seed, the tigrina mutations to the offspring of dry seeds. The concentration of erectoides mutations to dry seed offspring may explain the conflicting results obtained in different years after treatments with densely and sparsely ionizing radiations (EHRENBERG and NYBOM, 1954; NYBOM, 1956; EHRENBERG *et al.*, 1959). Neutron irradiation was originally considered the most efficient agent in order to induce erectoides mutations. Recent data are to some extent contradictory. The water content of treated seed was not intentionally varied in previous experiments with the extraction of erectoides mutations in mind. Neutrons largely act independently of the water content. Therefore they cause equal rates of chromosomal disturbances and gene mutations in dry and moist seed offspring (the dose kept constant) (EHRENBERG, 1955). X-rays, on the other hand, induce higher rates in the offspring of dry than of normal and moist seed. In analogy, when normal or moist seed is irradiated, the neutron series will induce more erectoides mutations, relatively seen, than X-irradiation. In years when dry seed has been irradiated,

the reverse will take place. Neutron rates should be constant, whereas X-ray rates should decrease with increasing water content. The problem cannot be settled definitely, however, until the influence of seed moisture on the mutation of individual erectoides loci has been carefully studied.

Here a brief discussion is appropriate as regards the *rare morphological* mutants. In our paper of 1959 we pointed out that such mutants appeared to be more frequent after the use of chemicals than after ionizing radiations. Certainly, some mutants listed had arisen in radiation experiments, too; several, however, had not been accounted for previously. In Table 6 the corresponding mutants of 1959 and 1960 are listed. Refraining from details, we may state that the chemical mutagens have also these two years induced some mutants not previously found in material obtained after treatment with ionizing radiations.

The high rate of ethyleneimine-induced rare mutants also in the last two years is a most interesting fact. Actually, they comprise twice as high a rate, relatively seen, as that obtained after irradiation, and they are decidedly more frequent than after treatments with glycidol or ethyl methanesulfonate. If we also include the short-awned mutants, which are not specially uncommon, the rates of the methanesulfonates series, too, give higher values than the ionizing radiations do. In fact, the statistical differences between EI, on one hand, and the radiations, on the other, give P-values of $0.02^{*(*)}$ and approximately $0.001^{**(*)}$, respectively. The corresponding comparison between the sulfonates and the radiations gives an indication of a difference only when the short-awned mutants are included ($P=0.05^{*}$), but the data are not strictly comparable. If the 1960-data are used there is no definite difference.

The occurrence of mutations with *lemma-like glumes* ("macrolepis") is interesting. In 1959 and 1960 eight cases were found in the X_2 offspring when chemical mutagens were used, none in the case of ionizing radiations. All mutations of this type belong to one single locus. The matter will be followed up in special experiments.

In addition to these morphological mutants there are a series of physiological changes (Table 7). Most interesting are the *early* mutants, both of the drastic and the modifying type (GUSTAFSSON *et al.*, 1960). They are rather frequent and easy to select owing to their enhanced heading time. Another group consists of *straw-stiff, erect* types. These are generally inferior in yield. Some, however, approach the mother line in production. Mutants with *irregular spikes* form transitions to the morphological group. Many have highly deformed ears but set

TABLE 6 a. *Morphological mutants.*

(X_2 1959, 1960) (*cf.* SMITH, 1951; NYBOM, 1954.)

Mutagenic agent	Types of mutation	No. of rare mutations	
		excl. short-awned	incl. short-awned
Neutrons (1959—1960)	12 erectoides 9 "bright-green"		
	4 short-awned 1 scirpoides 1 mutant with bent first internode 1 triaristatum 1 mutant with acute lemmas of side florets 1 nudum 1 mutant with smooth awns	6	10
X-rays (1959—1960)	13 erectoides 9 "bright-green"		
	5 short-awned 1 densinodosum 1 uniculmis 1 scirpoides 1 ligule-less 2 "accordion rachis" 1 intermedium 1 mutant with acute lemmas of side florets	8	13
Glycidol (1959—1960)	15 erectoides 5 "bright-green"		
	1 short-awned 1 "accordion rachis" 1 six-row mutant 1 "viviparum"	3	4
Ethyleneimine (1959—1960)	47 erectoides 47 "bright-green"		
	27 short-awned 2 uniculmis 1 mutant with bent tillers 3 densinodosum 2 "gigas" mutants		

Mutagenic agent	Types of mutation	No. of rare mutations	
		excl. short-awned	incl. short-awned
	2 mutants with brown stems 1 ligule-less 4 auricle-less 1 mutant with bent first internode 4 anthocyanin-free 1 mutant with branched spikes ("compositum") 4 intermedium 2 semideficiens 4 triaristatum 5 lemma-like glumes 3 with "double florets" 3 mutants with acute lemmas of side florets 1 "grand-pa" (MARTINI and HARLAN, 1942) 1 nudum 1 "viviparum"	45	72
Ethyl and propyl methanesulfonate (1960)	43 erectoides 27 "bright-green" 23 short-awned 2 scirpoides 1 "open dune-type" (growth habit like in dune ecotypes of *Hieracium umbellatum*, TURESSON, 1922) 1 mutant with brown stems 1 ligule-less 2 mutants with bent tillers 1 mutant with curved first internode 1 anthocyanin-free 1 "accordion-rachis" 1 intermedium 1 semideficiens 1 mutant with "double florets" 3 triaristatum 2 mutants with acute lemmas of side florets 3 lemma-like glumes 1 mutant with opposite kernels 1 mutant with brown kernels	23	46

TABLE 6 b. *Condensed data of Table 6 a.*

Mutagenic agent	No. of viable mutants	Per cent rare mutants	
		excl. short-awned	incl. short-awned
Ionizing radiations (1959—1960)	180	7.8	12.8
Glycidol (1959—1960)	48	6.3	8.3
Ethyleneimine (1959—1960)	313	14.4	23.0
Ethyl and propyl methanesulfonate (1960)	237	9.7	19.4
Sum	778	10.9	18.6

seed. Another transition type consists of the *lax-eared* mutants. Some of these, with oblong kernels, are characterized by the occurrence of 5 stamens (v. WETTSTEIN *et al.*, 1959). A trained worker can easily select mutants of this singular flower structure. The remainder of physiological mutants isolated in the X_2 generation consist of more or less abnormal types, often fungus-susceptible, deformed and low-yielding. Some are short- or dense-eared, light-coloured, late, semi-nudum, round- or oblong-kernelled, have wrinkled awns etc.

3. X_3 analysis of viable mutants

A definite judgment of induced aberrations is possible only after a study of several generations. Their characteristics must be evaluated and their mode of segregation analysed, before they are discarded, or included in the mutant collection or, even, propagated for yield trials. In our experiments, chlorophyll lethals are no longer followed up, with the exception of aberrants suitable in linkage tests or in anatomical and physiological studies. Dwarfs and other lethals are generally discarded in the X_2 generation, except for strikingly deviating types. Progenies containing "translocation sterility" are selected to a limited number for model studies, and so are progenies with "recessive sterility". Deformities of various kinds are kept, insofar as they are of special interest. Of viable mutations almost everyone is followed to further generations. A systematic hybridization work sets in for the localization of the genes and for the determination of the number of loci responsible for special

TABLE 7. *Physiological mutants (X_2: 1959, 1960).*

Mutagenic agent	Types of mutation
Neutrons (1959—1960)	17 early mutants 7 straw-stiff, erect mutants 11 lax-eared 7 mutants with irregular spikes 2 mutants with rounded kernels 1 broad-leafed 12 "others"
X-rays (1959—1960)	20 early mutants 7 straw-stiff, erect mutants 14 lax-eared mutants 4 mutants with irregular spikes 1 semi-nudum 1 late mutant 10 "others"
Glycidol (1959—1960)	7 early mutants 2 straw-stiff, erect 5 lax-eared 2 semi-nudum 1 rounded kernels 1 broad-leafed 1 large-kernelled 5 "others"
Ethyleneimine (1959—1960)	49 early mutants 10 straw-stiff, erect 32 lax-eared 10 with irregular spikes 3 seminudum 3 rounded kernels 1 oblong kernels 3 broad-leafed 3 late 33 "others"
Ethyl and propyl methanesulfonate (1960)	20 early mutants 3 straw-stiff, erect 35 lax-eared 12 with irregular spikes 5 seminudum 2 rounded kernels 1 oblong kernels 1 broad-leafed 10 late 32 "others"

TABLE 8. *X_3 analysis of viable mutants, selected in X_2.*

(X_2: 1958 and 1959, X_3 1959 and 1960.)

Mutagenic agent	Type of mutation and number of cases in X_3					Recovered cases in X_3
	Erectoides	Bright-green	Early	Others	Sum	
Neutrons	9	5	4	37	55	69 %
X-rays	12	8	9	33	62	68 %
Ethylene oxide	5	4	3	25	37	69 %
Epichlorohydrine	—	2	1	1	4	57 %
Glycidol	6	3	1	7	17	94 %
Diethyl sulfate	1	2	—	9	12	100 %
Ethyleneimine	46	32	25	171	274	86 %
Recovered cases in X_3	100 %	97 %	50 %	79 %	79 %	

characteristics (like erectoides, bright green, short-awned, six-rowed, intermedium, lemma-like glumes, bracteatum, etc.). As mentioned previously, some of these mutations are caused by changes in a great many loci, others by changes in few loci, or just one single locus.

Table 8 presents the data with regard to the viables isolated in 1958 and 1959 and then controlled in the X_3 generations 1959 and 1960. About 80 per cent of all mutations were recovered. Erectoides types, bright-greens and other clear morphological mutations, like six-row types, mutants with lemma-like glumes, without ligulae or auricles, are recovered to almost 100 per cent. The physiological changes, with regard to earliness, lateness, stiffness of straw, are recovered considerably less. This is exemplified in the table by the early mutants, 86 probable cases of which were selected in the X_2 generation. Only half of them were identified next year. This does not mean that all the remaining 43 cases were erroneously classified. The two years 1958 and 1959, as well as 1959 and 1960, were strikingly different as regards climatic conditions. An early mutant, say in 1958, may turn out not to be early in the next year, owing to different reaction modes of mother line and mutant. Many physiological properties fluctuate greatly from year to year. This holds true, *inter alia*, for earliness and lodging resistance.

There is another source of error. When ionizing radiations are applied, a certain sterility appears already in the first generation. It is followed in the second generation by the occurrence of partial sterility due to chromosome rearrangements. This hampers the selection of cer-

tain mutations, especially slight ones. Moreover, chromosome translocations (and possibly inversions) are themselves often associated with minor physiological changes (due to position effects or mutations at the breakage points), which interact with (intensify or counteract) the effects of concomitant mutations. The recovered mutants amount to approximately 70 per cent after X-ray and neutron treatment, but to approximately 85 per cent or more with ethyleneimine, glycidol and diethyl sulfate, which, relatively seen, cause less chromosome breakage and rearrangement and consequently less partial sterility than that induced by the radiations. In fact, the difference in behaviour between ionizing radiations and chemical mutagens (Table 8) is significant on a P-level of 0.001.

At least 43 cases of early mutants, selected according to enhanced heading time, still appeared in the X_3 generation. They comprise drastic as well as modifying types according to the classification of GUSTAFSSON *et al.* (1960). It can safely be assumed that some of them will show a full productivity, possibly with a further increase of lodging resistance, due to a parellel change in internode structure.

Straw-stiff mutants of the modifying type are rather easily selected, too. They are less readily recovered in the X_3 generation (to approximately 35 per cent). No doubt this depends on the fluctuation of the climatic conditions, since in one of two subsequent years there may occur an extreme lodging, in the other year very little lodging. Stiff mutants often decrease yield, or — *vice versa* — mutants decreasing productivity often seem to be more lodging resistant than their mother line. In such instances the selection primarily considers a decreased productivity. The mutants then appear to be resistant to lodging. This is in contrast to numerous erectoides and early mutants, which keep their productivity level and simultaneously increase their lodging resistance. There is no doubt that a planned selection for a changed internode structure (and for an increase in resistance to ear breakage) by means of mutations will contribute to the improvement of commercial varieties. Recent work in the Pallas variety of barley (resulting from a mutation in locus *ert-k*, BORG *et al.*, 1958) actually indicates that high-productive drastic mutants can be further improved by mutations with minor effects.

4. Control material

Every year a large control material is bred, both for greenhouse and field tests. Field controls are important for the critical analysis of in-

duced variation. In the years 1958—1960, 2,928 spike progenies of control material were inspected in the same routine manner as the treated material. They produced 5 chlorophyll mutants (0.2 %) and 39 cases of "translocation sterility" (1.3 %). The latter figure seems high, but many of these cases are modifications, in conformity with previous analyses. No dwarfs or viable mutations were encountered.

VI. CONCLUSIONS

The aim of this study was to compare the genetic effects of ionizing radiations and chemical mutagens, especially with regard to the induction of viable mutants.

(1) *X_1-analysis.* — The *first generation effects* studied comprised chromosome fragmentation and translocation, as well as X_1 lethality and fertility. Chromosome aberrations are generally abundant, especially in the first mitotic cycle of dormant seeds, after the treatment with high doses of ionizing radiations. They are less common with chemicals like ethyleneimine, and then they first appear in the second or later cycles of mitosis, or are almost absent at low and medium doses with genetically powerful alkanesulfonic esters. Such cytological analyses have been performed by J. and M. MOUTSCHEN (1958, unpubl.), FAVRET (*cf.* his paper of 1960 b), NATARAJAN (unpubl., *cf.* EHRENBERG, 1960 b). Recently HEINER *et al.* (1960) further stressed the contrast in cytological efficiency between chemical mutagens and ionizing radiations originally pointed out by EHRENBERG *et al.* (1956 a and b). The X_1 sterilities, produced after mutagenic treatments, reach high values with neutron irradiation and ethyl methanesulfonate. With X-irradiation and ethyleneimine treatment, on the other hand, the sterility values seldom surpass 50 per cent.

(2) *X_2-analysis.* — The *second generation effects* especially concerned chlorophyll mutations in greenhouse tests and various types of aberrations in field tests. If ionizing radiations are used, the rates of *chlorophyll mutations* in greenhouse tests approach some 8 or 10 per cent or may, under certain experimental conditions, reach values of 13 to 14 per cent. After treatment with ethyleneimine, the corresponding figures are 25 to 30 per cent, and with alkanesulfonic esters values of 50 or 60 per cent are obtained. The highest percentages are generally reached in experiments using fairly sterile X_1 offspring.

For each per cent increase of the induced mutation rate there is a parallel rise in X_1 sterility. The *average* rise in sterility amounts, (according to one special method of calculation, p. 249),

for neutrons	to 7.5 per cent
„ X-rays	„ 5.1 „ „
„ ethyleneimine	„ 1.3 „ „
„ ethyl methanesulfonate	„ 1.1 „ „

The *field aberrations* studied comprised chlorophyll mutations and other lethal and semilethal types, furthermore so-called translocation sterility (TS) and various kinds of viable mutants. The rates of the field aberrations considered in an X_2 generation reach values above 100 per cent, calculated per spike progeny, or above 400—500 per cent calculated per plant progeny. The very high values are obtained with chemical mutagens, especially in samples with a fairly pronounced X_1 sterility.

The central problem of this investigation deals with the *viable mutants*. The maximum rate of any X_1 offspring, calculated on spike basis, amounts to 5—9 per cent if ionizing radiations are used, about 18 per cent if ethyleneimine is used and close to 30 per cent after ethyl methanesulfonate treatment. Mean rates are at their highest in experiments using offspring of fairly high X_1 sterility. However, even in the case of offspring from fully fertile X_1 plants, chemical mutagens produce twice as high rates as do the ionizing radiations. To extract viables free or almost free from concomitant lethals and rearrangements (*cf.* STADLER, 1930) selection work should, in the case of radiations, concentrate on fertile X_1 plants, despite the fact that the rates of viables are low. For a corresponding low proportion of lethals and rearrangements in the case of EI and EMS, the selection work of viables can utilize the mutation rates, approximately ten times as high, occurring in the offspring of more or less sterile X_1 plants.

(3) *X_2-analysis.* — The viable X_2 aberrants are generally followed to *the third generation.* Most morphological mutants recur. Minor changes, for instance with regard to earliness or lodging resistance, are recovered to less extent. This often depends on the contrasting climatic conditions of subsequent years. Nonetheless, in 1959 and 1960 no less than 43 mutants with enhanced earliness were identified, some of which no doubt fully productive and, in addition, less lodging than their mother lines (*cf.* GUSTAFSSON *et al.*, 1960). The translocation sterility itself, possibly also position effects or slight mutations at the points of chromosome breakage, may counteract the recovery in the X_2 generation of minor physiological mutants.

(4) *Differences in mutation spectra.* — With regard to the spectrum

of *chlorophyll mutations* the conclusions drawn by EHRENBERG *et al.* (1959), as well as v. WETTSTEIN *et al.* (1959) have been confirmed. Ionizing radiations and chemical mutagens act differently with regard to the induction of albina, viridis and rare chlorophyll mutations. There is some indication that ethyleneimine and ethyl methanesulfonate, too, produce different mutation spectra.

The mutant proportions of various types of *field aberrations* differ considerably for the two kinds of mutagens. The occurrence or absence of chromosome rearrangements is directly reflected in the amount of "translocation sterility". Partial X_2 sterility ("semisterility") is chiefly caused by translocations (or other gross chromosome changes) in the case of ionizing radiations. After treatment with chemical mutagens, like EI and EMS, true translocation sterility due to segmental interchange is realized to less extent. There is in the case of chemical mutagens a lower ratio of progenies with "translocation sterility" to progenies where viable mutants are isolated. This is another change in mutation spectrum distinguishing radiations and chemicals.

Some regularities exist with regard to morphological mutants, too. In the chemical treatments of the years 1958—1960 a great number of X_2 mutants appeared not found in the simultaneous radiation series. Ethyleneimine was especially efficient in this respect. After treatments with this compound there arose definitely more rare mutants than after any kind of radiation. The difference is statistically significant. This implies that there is a possibility of changing the mutation spectrum with regard to viables, too. In fact, ethyleneimine seems even more useful than the sulfonates tested.

(5) *The induction of specific mutations.* — In a recent publication FAVRET (1960 b) compared the action of X-rays and ethyl methanesulfonate with regard to four chlorophyll loci of barley. While X-irradiation caused all four loci to mutate, EMS-treatment affected only one. Consequently, there is a specificity of this chemical on individual loci. The fact that radiation worked on all loci, may depend on losses of the segments, containing the loci in question, as well as on mutations in the loci themselves. Under certain circumstances even different kinds of radiations are selective in their action, as shown by neutron *contra* X- or γ-radiation in the case of the erectoides locus *a*, where no mutations have as yet arisen with neutrons, although rather frequently with X- or γ-rays. Another indication of a specific action of different mutagens was published by FAVRET (1960 a) regarding mildew resistance. Mutations from susceptibility to resistance arose under the influence of

ionizing radiations, but not after treatments with ethylene oxide, although in parallel series both agents induced chlorophyll mutations at similar rates. Mildew-resistant mutations appear to be the result of two-hit phenomena, since they are formed proportionally to the square of the dose, in contrast to the chlorophyll mutations, which increase linearly. Genes for mildew resistance are largely concentrated to an "isophenic" segment of chromosome 5 and possibly, in accordance with FAVRET's conclusions, conditioned by a series of small duplications. Whatever the mechanical explanation of such mutations, there is apparently a difference in action between the ionizing radiation and the chemical mutagen tried. These data indicate that we now possess methods for the induction of specific viable mutations.

Another finding of possible significance relates to the accumulation of erectoides mutants in the offspring of dry X-irradiated seed in 1960. Seed of 7—10 per cent water content gave erectoides mutants at a rate of 3.4×10^{-6} per r-unit and spike progeny. This value is more than 30 times higher than the corresponding figure for moist seeds of 15—20 per cent water content (0.1×10^{-6}). The discrepancy between mutation rates in different years may depend on such influences. This is possibly the case, too, with regard to the conflicting results obtained using densely and sparsely ionizing radiations. A similar influence, probably of metabolic conditions, on the mutation rates was postulated by D'AMATO and GUSTAFSSON (1948) in their work dealing with the alboxantha and tigrina types of chlorophyll mutations.

(6) *The usefulness of different mutagenic agents.* — A few words will be said about the relative value of individual mutagenic agents in mutation research and plant breeding. Under the impression of the high efficiency of the mutagenic chemicals now available, one may possibly consider the ionizing radiations obsolete and unnecessary. Such a conclusion is not so far justified, however, as is clear from the results outlined under 4 and 5. Chromosome translocations, the consequence of chromosome breakage, are especially abundant in radiation experiments and will often lead to new systems of gene balance, expedient in the utilization of newly arisen mutations. Deficiencies, also common with ionizing radiations, are valuable for the continuous conversion of wild or semi-wild species to the domesticated state. Duplications, arising either directly, or indirectly after an appropriate handling of translocations, will be helpful in many matters, for instance in the acquirement of fungus resistance or in the breeding of special enzyme systems (HAGBERG, 1960). Actually, "chromosome engineering" by

means of chromosome breakage and translocation is most easily effected by ionizing radiation, until definite chemicals are found permitting a precise induction of breaks and rearrangements (which may ultimately happen). Even then the ionizing radiations will certainly still be useful. There has been some dispute concerning the use of X-rays or neutrons (or both) in mutation research. We definitely want to state that sparsely ionizing radiations cannot be omitted as mutagens. This is evidenced, *inter alia*, by the radiation induced mutations of the ert-locus *a* in barley. Neutrons, on the other hand, are quite useful for many purposes, since they act directly on the genetic material, in contrast to X- or γ-radiation, which *via* more or less indirect events produce genetic changes. Owing to this fact, neutron effects are less influenced by differences in physical, chemical and metabolic conditions. Also, the differential action of neutrons and X-rays on X_1 lethality and fertility argues for a parallel use.

With regard to chemical mutagens, there is much work ahead, until individual mutagens can be definitely evaluated. Mutagens causing chromosome breakage in a precise and predictable manner, up to now rather inefficient, must be improved. On the other hand, the alkanesulfonic esters are most efficient mutagens, although they are not especially effective in chromosome breakage. With regard to morphological mutants, there is some indication that ethyleneimine produces higher rates of rare types than do the methanesulfonates. Both these groups of compounds have to be further examined and compared in parallel experiments.

In spite of the relatively high mutation rates obtained with such chemicals as ethyl methanesulfonate and ethyleneimine, and in spite of the breadth of the mutation spectrum with regard to rare mutants, especially of the latter compound, there are many indications that these and other chemicals have an affinity to the gene material which is more restricted to certain regions than that of the ionizing radiations. We have already mentioned FAVRET's observations (*l.c.*) that, in contrast to ionizing radiations, ethylene oxide was unable to induce mutation for mildew resistance in barley, and that ethyl methanesulfonate produced a change in only one of four albina genes, all of which were sensitive to X-rays. Such findings are in agreement with the fact, that in contrast to the random distribution of breakage or exchange points after treatment with ionizing radiations, most chemicals seem to induce aberrations at points more or less concentrated to certain chromosome regions. In several cases (barley, peas and other, especially diploid

plants) it is possible to reach many times higher overall mutation rates if certain chemicals are used than can be obtained by irradiations. One ought to be careful not to overestimate the usefulness of the chemicals. Especially when new species or varieties are taken up for mutation research, ionizing radiation should always be included in the experiments, since the randomness in their action implies that they, in contrast to the chemicals, are capable of inducing a variation in most genetically controlled properties. (A possible restriction to the usefulness of such coarsely acting agents like the ionizing radiations may exist, when a locus is linked with or consists of some gene material, the intactness of which is of vital importance to the cell.) The high mutagenic efficiency of some chemicals, on the other hand, makes it advantageous also to include such agents in experimentation with new materials (*cf.* EHRENBERG, 1960 b).

Finally, it may appear useful in mutation research and plant-breeding to combine different types of mutagens, for instance radiations and chemical compounds, and to treat one original material repeatedly, thereby successively increasing variation. Mutation rates of thousand per cent, or much more, can easily be accumulated in such sets of experiments. However, the parallel induction of viable and lethal mutations in one and the same chromosome or chromosome segment (STADLER, *loc. cit.*) must be carefully considered. The optimum level of induced mutation rates and types has to be worked out for different mutagens, as well as for different organisms.

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SUMMARY

(1) The aim of the present study was to compare the genetic effects of ionizing radiations and chemical mutagens, especially with regard to the induction of viable mutants.

(2) The chief mutagens used consisted of neutron and X-irradiation, as well as treatments with glycidol, ethyleneimine and ethyl methanesulfonate.

(3) Mutations were analysed in greenhouse and field tests. The greenhouse mutants ("chlorophyll mutants") amounted to 4,000 cases in the

years 1958—1960. The field aberrations consisted of 2,400 chlorophyll mutants, 650 dwarfs and other lethals, 5,200 cases of so-called translocation sterility and more than 1,000 cases of viable mutants. The total sum of X_2 aberrations, analysed in the field, was 9,300, in circa 21,500 spike progenies.

(4) Neutrons and X-rays generally produce rates of greenhouse mutations up to 8 or 10 per cent, or under special experimental conditions up to 13 or 14 per cent. Using ethyleneimine, rates of 25 to 30 per cent are reached and using ethyl methanesulfonate, rates of 50—60 per cent.

(5) The spectrum of chlorophyll mutations varies with the kind of mutagen employed. Ionizing radiations produce, relatively seen, more albina and less viridis and rare chlorophyll mutations, chemical mutagens less albina and more viridis and rare mutations.

(6) With regard to field aberrations there is a striking difference in mutation spectra, too. Cases of "translocation sterility" are less abundant, relatively seen, in the case of ethyleneimine and ethyl methanesulfonate treatments than in the case of irradiation. The ratios of offspring showing translocation sterility to offspring with viable mutants are high applying neutrons and X-rays, but low applying ethyleneimine and ethyl methanesulfonate.

(7) The central part of the study concerns the rates and types of viable mutants. The maximum X_2 rates found, calculated on spike basis, amount to 5—9 per cent using ionizing radiations, 18 per cent using ethyleneimine and close to 30 per cent using ethyl methanesulfonate. Mean rates are highest in samples with a profound X_1 sterility.

(8) Ethyleneimine is especially efficient in inducing rare morphological mutants. After treatments with this compound there arose more rare mutants, relatively seen, than after any kind of radiation or after the treatment with ethyl methanesulfonate.

(9) In general, viable mutants are followed to the X_3 generation. Most morphological mutants recur. Small changes of earliness and lodging resistance, on the other hand, are recovered less frequently. Nonetheless, in 1959 and 1960 43 cases of mutants showing an enhanced earliness were identified, some of them with drastic, some with slight effects.

(10) The use of one densely and one sparsely ionizing radiation (neutrons and X-rays, or γ-rays) is advisable in mutation work in order to cover the entire range of radiation induced mutations. Similarly, the use of at least two chemical agents is recommended, both of them highly efficient in causing gene mutation, *viz.* ethyleneimine and ethyl meth-

anesulfonate. Chromosome-breaking chemicals, rearranging the chromosomes in specific ways and more efficient than the ones now used (8-ethoxycaffeine and myleran), should be worked out.

(11) Problems relating to the capacity of radiations and chemicals in causing specific gene mutations are briefly discussed.

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RADIATION SENSITIVITY OF PINE SEEDS OF DIFFERENT WATER CONTENT

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I. INTRODUCTION

THE aim of this experiment was to obtain information about the germination and seedling growth of pine seeds of varying water content irradiated by γ-rays and to find out favourable conditions for inducing mutations.

This kind of work has long been in progress on agricultural plants. GUSTAFSSON (1947), following STADLER (1928, 1930), reported that the sensitivity of water-soaked barley seeds to X-ray treatment was positively related to their water content. EHRENBERG (1953) found that an increasing sensitivity of barley seeds to X-rays appears as a consequence of decreasing water content. CALDECOTT (1954, 1955) has shown that barley seeds with 8—16 % water content are more resistant to X-ray damage than those with either a lower or higher water content. ABRAMS and FREY (1957) also found the same phenomenon using oat seeds. EHRENBERG *et al.* (1958, 1959) following up previous experiments (1953, 1954) studied the radiation effects on seeds of different water content in relation to the mechanism of action of radiations. Measuring electron spin resonance values of the samples, they explained how water plays its role in connection with free radicals produced by the irradiation.

With respect to forest tree seeds few papers have been published on this problem, although it certainly is important to forest tree breeding to gain a better understanding of the response of tree seeds to ionizing radiation.

1. Material

Seeds of Japanese red pine (*Pinus densiflora* SIEB. et ZUCC.) were used in this experiment. In 1959, the cones were collected from seven trees individually at the Ichinoseki red pine experiment field, Iwate

Pref., Japan. As GUSTAFSSON and SIMAK (1958) reported that the radiation sensitivity of pine depends on the developmental stage of the embryo, only fully developed seeds, embryo class IV, were used following selection by the method of X-ray photography devised by SIMAK and GUSTAFSSON (1953). Although it is preferable to use seeds of individual trees, unfortunately there were no single trees from which a sufficient number of seeds could be collected to cover the entire experiment. So, seeds from seven trees were mixed as follows: from each tree 15 or 16 seeds were taken and put together making 108 seeds as a treatment unit. In sowing $34 \times 3 = 102$ seeds were used out of 108 seeds. The seed weights are given in Table 1.

2. Treatments

Seven different doses and six different water contents were applied in combination. Irradiation doses were 0, 3, 6, 9, 12, 15 and 18×10^3 r. To obtain seeds of different water contents, they were equilibrated for one week in air currents of relative humidity 98, 85, 62, 30, 12 and 0 % respectively, secured by passing air through KOH solution or conc. H_2SO_4 (EHRENBERG, 1955). The equilibration was done at the Institute for Organic Chemistry and Biochemistry, Stockholm University. Seeds were adjusted to the water contents shown in Table 2. Water content of the seeds was determined by comparing their fresh weights with weights of samples after drying at 105° C for one day.

The seeds were irradiated at the same institute by γ-rays from a 3 000 C Co^{60} γ-source in the same small plastic tubes in which they were equilibrated. Dose rate of the γ-source was 8 333 r/min. So, 21, 43, 64,

TABLE 1. *Seed weights.*

No. of tree		Weight of 1000 seeds (embryo class IV) (g)	Number of seeds taken for the experiment	
1	Ichinoseki C	8.196	15×42	630
2	D	7.110	15×42	630
3	E	10.312	16×42	672
4	F	7.412	16×42	672
5	G	9.794	16×42	672
6	H	7.651	15×42	630
7	I	9.443	15×42	630
Total number			108×42	4,536
Number of sown seeds			102×42	4,284

TABLE 2. *Water content of seeds.*

Solution	Expected relative humidity of air (%)	Water content of the seeds after equilibration (per cent of dry weight)
A KOH 5 gr/100 ml water	ca. 98	16.3
B 20	85	12.7
C 40	62	8.8
D 70	30	7.5
E 100	12	6.1
F conc. H_2SO_4	0	2.2

86, 107, 129 seconds of irradiation were applied for doses of 3, 6, 9, 12, 15 and 18×10^3 r respectively. The irradiation times were so short that no conspicuous change in water content of the seeds took place during the irradiation.

3. Experimental design

There were three replications, comprising 102 (34×3) seeds per treatment unit. Immediately after irradiation seeds were sown in sand specially prepared in stainless steel boxes (size, $29 \times 39 \times 11$ cm) at 0.5 cm depth. The germination beds were kept moist during the whole experimental period by the aid of water-absorbing layers of filter paper. The intensity of the continuous illumination was approximately 1 000 Lux at the surface of the germination beds. Room temperature was kept between about 20 and 25° C. Every day germinated seedlings were counted and marked. The experiment was terminated 60 days after sowing, and living plants were cut for measurement.

II. RESULTS

The following criteria were applied as indicators of radiation effects on irradiated pine seeds and seedlings that arose from them.

a. Germination percentage
b. Survival percentage
c. Plant development
   i Plant height
   ii Hypocotyl length
   iii Cotyledon length
   iv Dry matter weight
d. Stages of dead plants

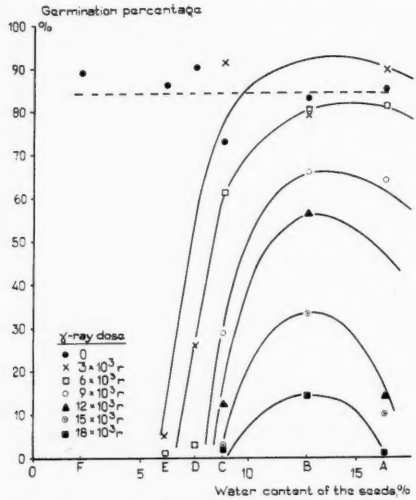

Fig. 1. Water content of seeds and germination percentage after various γ-ray doses.

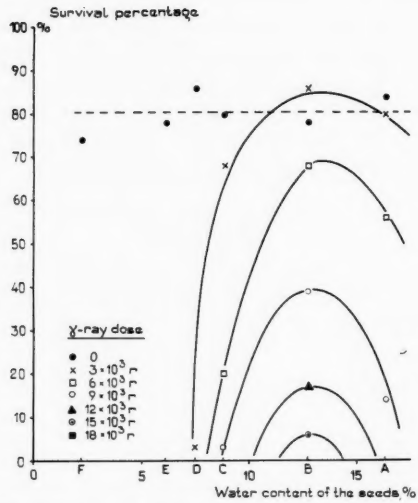

Fig. 2. Water content of the seeds and the survival of seedlings after various γ-ray doses.

a. *Germination percentage* (Fig. 1)

There were large differences in germination percentages among irradiated seeds of differing water contents. The highest germination was obtained in seeds with 12.7 % water content. No irradiated seeds with the lowest water content, 2.2 %, germinated, while unirradiated seeds that had been dried identically germinated to 89 %. Seeds of 6.1 and 7.5 % water contents were also rather sensitive to the radiation and showed marked inhibition of germination.

b. *Survival percentage* (Fig. 2)

Survival percentage was calculated by

$$\frac{\text{Number of living seedlings 60 days after sowing}}{\text{Number of germinated seeds in the same period}} \times 100$$

As shown in Fig. 2, there were no survivors from seeds irradiated while they had water contents of 2.2 or 6.1 %. The highest survival percentage following any dose of radiation was among seedlings resulting from seeds irradiated while containing 12.7 % water. The survival percentage was a little higher in seedlings from irradiated seeds of 16.3 than 8.8 % water content. Apparently seeds irradiated while their water content is 12—14 % yield the highest per cent survivors.

c. *Plant development* (Figs. 3, 4, 5, 6)

There were no survivors in treatments where seeds received higher doses than 12 000 r, unless the seeds were irradiated while their water content was 12.7 %. With lower doses the height of survivors was maximal for seeds irradiated at 12.7 % water content (Fig. 3).

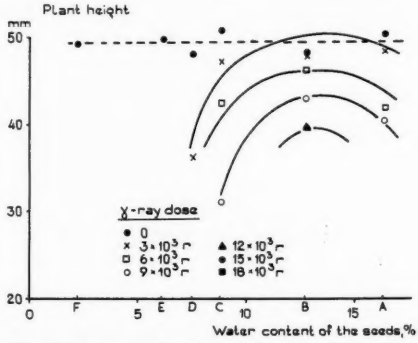

Fig. 3. Water content of the seeds and plant height after various γ-ray doses.

As shown in Figs. 4, 5, and 6, hypocotyl length, cotyledon length and dry matter weight showed the same trend as plant height in response to the treatments.

d. *Stages of dead plants* (Fig. 7)

In rating dead plants the following classification was used:

TABLE 3. *Developmental stages.*

Stage	Descriptions of stages
N	Seedlings with normal appearance in all features
I	Germinated seeds, the seed scale observable, but hypocotyl not visible
II	Seed scale still attached to the short, frequently thickened hypocotyl, no cotyledons visible
III	Same as II, but lower part of the cotyledons visible
IV	Short and thick plants without seed scale, similar in appearance to colchicine treated seedlings

In Fig. 7, the per cent survivors and "stages of the dead plants" is shown for each treatment. These stages are described in the figure and in Table 3. Numerators and denominators indicate the number of dead

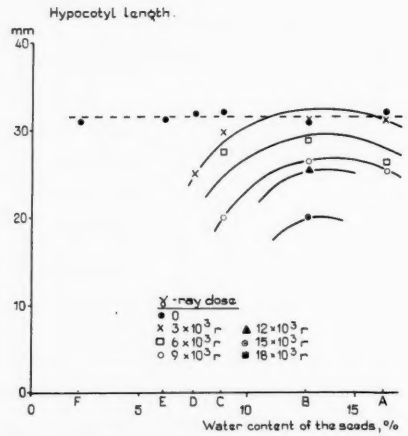

Fig. 4. Water content of the seeds and length of hypocotyl after various γ-ray doses.

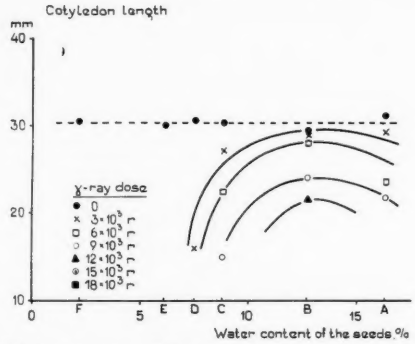

Fig. 5. Water content of the seeds and length of cotyledons after various γ-ray doses.

plants and the number of germinated seeds in each treatment, respectively. In unirradiated controls there were 13—20 dead plants and more than 50 % of them were in stage I. Most of the seedlings arising from irradiated seeds of lower water content and from highly irradiated seeds died in stage I. On the other hand, seedlings grown from seeds with high water content during irradiation with lower doses, died very often in stage N, and more often in stage II or III than seedlings from unirradiated seeds. Some seedlings of stage IV, similar in appearance to

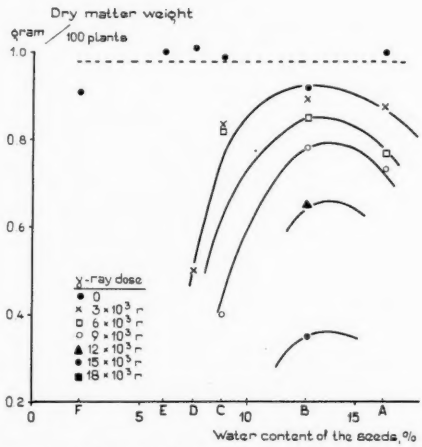

Fig. 6. Water content of the seeds and dry matter weight of the seedlings after various γ-ray doses.

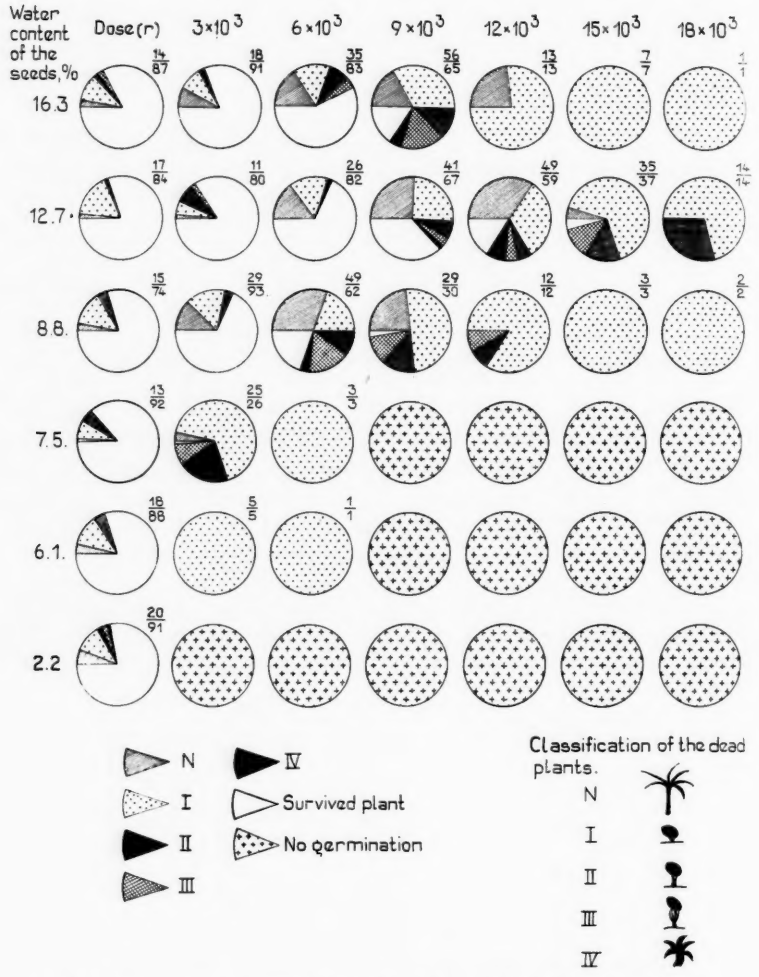

Fig. 7. Frequency of surviving plants and dead plants in various stages.

colchicine treated plants, were observed in the following combinations of dose and water content of seeds: 9 000 r, 16.3 %; 12 000 r, 12.7 %; 9 000 r, 12.7 %; and 6 000 r, 8.8 %; and most of them died before the termination of the experiment.

III. DISCUSSION

This experiment was carried out to get some information about the radiation sensitivity of pine seeds (*Pinus densiflora* SIEB. et ZUCC.) with different water contents. The highest resistance to γ-radiation was found in seeds with 12.7 % water content, and higher or lower water content induced higher radiation sensitivity. This result is similar to results obtained by others with different kinds of seeds and radiation sources.

EHRENBERG and NYBOM (1954), in barley, studied the neutron and X-ray induced inhibition of plant growth after seeds of differing water contents were irradiated. They found that ensuing plant growth was very much reduced when seeds of 8.6 % water content were X-irradiated. Highest plant growth was obtained when X-irradiated seeds were brought to 18.1 % water content before irradiation. EHRENBERG (1955 a, 1955 b) has published several papers on the relationship of water content and radiation sensitivity in barley seeds. He reported that the effectiveness of sparsely ionizing radiation decreases with increasing water content of seeds, in the region of 7—20 % water content, and if the physiological state of the seeds is not changed, the moisture influence on the radiation sensitivity is reversible. As mentioned in the introduction, CALDECOTT (1954, 1955) also found that barley seeds with 8—16 % water content were most resistant to X-ray damage. He suggested that the higher sensitivity of seeds with lower water content is indeed a function of water content, because when seeds that had been stored at a high relative humidity were desiccated, their sensitivity to X-rays increased again.

EHRENBERG, A. and EHRENBERG, L. (1958), EHRENBERG, L. (1959), and SPARRMAN *et al.* (1959) have investigated the influence of the water content of seeds on their radiation sensitivity with regard to the role of free radicals. They found that at very low water content the sensitivity of seeds is high, and sensitivity decreases to a minimum at 12—16—20 % water content (depending on the material). At still higher water content the sensitivity again increases. The same number of free radicals will be induced by a given dose of radiation (EHRENBERG, A., EHRENBERG, L. and ZIMMER, 1957). By measuring electron spin resonance of the irradiated seeds of *Agrostis stolonifera*, they found that free radicals produced by irradiation were quite stable and lasted several days in the driest seeds. With increasing water content the free radicals decay very rapidly to a semiconstant level. These authors also reported

that growth inhibition of the seedlings is proportional to this semiconstant level of free radical concentration for each water content (1955). For the increasing sensitivity of the seeds by a still higher water content (above 20 %), they suggested that the sensitivity may be increased by a new type of chemical reaction following the metabolic activity of the organism at this higher water content.

In our experiment with pine seeds based on germination percentage, survival percentage and plant development, there was the same relationship between water content and sensitivity as that reported for *Agrostis stolonifera* (SPARRMAN *et al.*, 1959).

In connection with the different stages of dead plants, there was a very high mortality in plants that arose from irradiated seeds, and many of these plants were classified as normal in their appearance. In barley (NYBOM *et al.*, 1952; EHRENBERG and ANDERSSON, 1954; EHRENBERG and SAELAND, 1954 a, cited from EHRENBERG, 1955 a) after X-irradiation of seeds, nearly all plants which develop their first foliage leaves are able to recover from injury and continue their growth to maturity, whereas after neutron irradiation many seeds produce normal or subnormal seedlings which then suddenly die. In pine, the result of γ-irradiation resembles the result of neutron irradiation rather than X-irradiation as described for barley (*cf.* Fig. 7, EHRENBERG and V. WETTSTEIN, 1955).

This experiment shows that Japanese red pine seeds of ca. 13 % water content have a minimum sensitivity to γ-radiation, and it seems favourable to apply doses of 9 000—12 000 r to seeds with this water content to obtain the maximum number of surviving plants showing effects of the irradiation.

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SUMMARY

Sensitivity to γ-radiation of Japanese red pine seeds with different water contents was studied in this experiment. Only seeds of embryo class IV (mature embryo) originating from seven trees were used. Seven

different doses and six different water contents were applied in combination. Doses were 0, 3, 6, 9, 12, 15, and 18×10^n r. Seeds were brought to 16.3, 12.7, 8.8, 7.5, 6.1 and 2.2 % water content by equilibration with air of varying humidity.

As the indicators of radiation sensitivity, the following criteria were applied:

a. Germination percentage
b. Survival percentage
c. Plant development
   i Plant height
   ii Hypocotyl length
   iii Cotyledon length
   iv Dry matter weight
d. Stages of dead plants

According to these measurements, the minimum sensitivity was found in seeds with ca. 13 % water content. Seeds with higher or lower water content were more sensitive. As shown in Fig. 7, irradiated seeds of higher or, especially, lower water content yielded very low germination percentages and high seedling mortality. For the purpose of securing X_1 plants of Japanese red pine, which show the influence of the irradiation, it seems profitable to bring seeds to a water content of ca. 13 % and to irradiate them with doses of 9 000—12 000 r.

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A SPECIFIC AND STRONG MUTAGENIC EFFECT OF N-NITROSO-N-METHYLURETHAN IN *OPHIOSTOMA*

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A strong mutagenic effect of N-nitroso-N-methylurethan (NMU) on *Vicia faba* and *Ophiostoma multiannulatum* has been briefly described in two previous reports (KIHLMAN, 1960; ZETTERBERG, 1960 b). In further experiments with *Ophiostoma* the mutagenic effect of NMU was qualitatively different from the effects previously obtained in forward-mutation experiments with this fungus, *i.e.* a different distribution (spectrum) of biochemical mutant types was produced.

Since 1946 Professor NILS FRIES has collected an extensive number of mutants from *Ophiostoma* by induction with ultraviolet light (UV), X-rays and different chemical mutagens. The mutant spectrum produced by NMU is compared with the spectra obtained by FRIES (1948 a, 1948 b, and personal communication).

Ophiostoma multiannulatum is an ascomycete. The wild type of the fungus can be grown in a medium containing a carbon source, a nitrogen source, inorganic salts and two vitamins, thiamine and pyridoxine. Strains with additional nutritional requirements can be obtained either spontaneously or after treatment with mutagens. With the methods used here (FRIES, 1948 b) it is possible to identify the additional requirements if they fall in any of the following classes: amino acids, nucleotide constituents, vitamins, reduced sulphur or fatty acids.

The treatments with NMU were performed by the following methods: Conidia from a culture grown in liquid medium for two days were filtered through cotton to remove fragments of hyphae. The conidia were centrifuged and washed twice in distilled water. They were then suspended in a phosphate buffer pH 7.0 at 22° C to which NMU (final concentration 10^{-4} M) had just been added. After 50 minutes the conidia were centrifuged down. The NMU solution was poured off and the conidia were resuspended in distilled water 60 minutes after the beginning

of the treatment. After repeated washing in distilled water the conidia were plated in complete medium and incubated at 25° C. The methods used for isolation of biochemical mutants are usually called "total isolation" and are identical with those described by FRIES (1948 b).

RESULTS

The results are summarized in Table 1, where also, for comparison, the results of recent experiments with UV and X-rays are shown (FRIES, unpublished).

NMU proved to be the most potent mutagen ever tried in *Ophiostoma*. In the first experiment the NMU-treatment killed 99.15 per cent of the conidia. Of the survivors 1145 cultures were isolated and 31 proved to be biochemical mutants, *i.e.* about 3 per cent. In the second experiment 99.4 per cent were killed; 855 were isolated; 34 were mutants, *i.e.* about 4 per cent. Irradiation with UV (2537 Å) or caffein treatment have sometimes given up to 2 per cent mutants at comparable survival. (FRIES, personal communication.)

The different mutant spectrum obtained with NMU was suspected to be accidental in the first experiment (ZETTERBERG, 1960 b). However, the same peculiar difference from the earlier spectra appeared when the experiment was repeated. Furthermore, by identification of the mutants some types were obtained never before isolated in *Ophiostoma*. A comparison between the NMU mutant spectrum and the mutant spectra obtained by FRIES (1948 a and unpublished) shows that the proportion of amino acid-less mutants was considerably higher in the NMU experiments. (See Table 1.)

Especially interesting are the new mutant types which appeared after NMU-treatment. Among the several thousands of mutants isolated in *Ophiostoma* histidine-less mutants have never been found. After NMU-treatment this type is quite common. The leucine-less mutant which appeared in the second NMU experiment is also new for *Ophiostoma*, and so is a mutant which has the peculiar physiological requirement of riboflavine *or* biotin.

Six of the amino acid-less mutants grew in minimal medium enriched with NZ-case and tryptophane, but did not grow when any one of 21 different amino acids was added to the minimal medium. Nor did they respond to addition of isoleucine+valine. Probably these mutants have complex requirements of amino acids and might be double mutants. There were also three mutants which grew only in complete medium,

TABLE 1. *Distribution of biochemical mutant types in Ophiostoma induced by treatment with N-nitroso-N-methylurethan (NMU), ultraviolet light (UV) and X-rays.**

Metabolite required	Number of mutants induced with			
	N-nitroso-N-methylurethan		UV	X-rays
	Exp. No. 1	Exp. No. 2	8 exp:s	16 exp:s
An amino acid	23	22	52	37
Arginine	6	2	10	5
Lysine	6	3	9	7
Methionine	2	5	33	25
Histidine	4	5	—	—
Leucine	—	1	—	—
Unknown	5	6	Other amino acids: 0	
Back-mutated	3	2		
Not identified	2	4		
A nucleotide constituent	4	7	59	31
Uracil	2	—	36	14
Hypoxanthine	1	6	18	14
Adenine	—	1	3	3
Guanine	—	—	2	—
Back-mutated	1			
A vitamin (growth factor)	1	3	14	9
Inositol	—	2	Not specified here.	
Riboflavine *or* biotine	1	—		
Back-mutated		1		
Reduced sulphur	1	—	—	—
A fatty acid	1	—	13	2
Unknown factor	1	2	—	—

* The data from UV and X-radiation are from unpublished experiments by N. FRIES.

but not in any of the media used for identification. These might require some compound which is present only in the complete medium used, or they might be double mutants with the required metabolites falling in different classes.

DISCUSSION

By treatment with alkali NMU can be converted into the alkylating agent diazomethane (PECHMANN, 1895). It is probable that this conversion also can occur *in vivo*. Diazomethane, ethyldiazoacetate and NMU

have been found to produce mutations in *Drosophila* (RAPOPORT, 1948). JENSEN *et al.* (1949) showed that diazomethane increased the back-mutation rate of an adenine-less mutant in the *Neurospora* back-mutation test. KIHLMAN (1960) found NMU to be a very potent chromosome breaking agent in root tip cells of *Vicia faba.* He also tested ethyldiazoacetate and N-nitroso-N-methylurea but found no radiomimetic effect of these compounds. Both NMU and N-nitroso-N-methylurea are used for the synthesis of diazomethane. LOVELESS (1951) did not find any chromosome breaks in root tips of *Vicia faba* after treatment with diazomethane.

Diazomethane is a very unstable compound. When added to a solution in which root tips are immersed it is probable that most of it is wasted in useless reactions before it reaches the chromosomes in the relatively inaccessible and large root tip cells of *Vicia faba.* When smaller cells, like *Neurospora* conidia, are treated with diazomethane the probability of mutagenic reactions should be greater.

The present results using NMU show it to be a more potent mutagenic agent than nitrogen mustard, which is another alkylating agent (FRIES, 1948 b). The low yield in FRIES's experiment and in an experiment recently performed by the present author does not allow a qualitative comparison between the mutagenic effects. Another alkylating compound, dimethylsulfate was far less mutagenic than NMU (ZETTERBERG, unpublished) and gave only 3 mutants out of 460 isolated cultures. The treatment killed 99.9 per cent of the conidia. It has to be kept in mind that the mutagenic effect, expressed as the percentage of survivors, should always be related to the degree of survival in the experiment, because the proportion of mutants increases with killing effect. It is easily understood that very lethal compounds are not necessarily effective mutagens. They may kill the treated cells by reactions with cell compounds which are not likely to interfere with the genetic mechanisms. KÖLMARK and WESTERGAARD (1953) have suggested some requirements of a chemical compound that are necessary for making it an effective mutagen. In summary the action of the mutagen should be preserved until it hits the target, whatever and wherever in the cell the target may be, *i.e.* the reactions started by the mutagen should affect the genes directly or compounds important for the integrity of the genes. The strong mutagenic effect of NMU might be due to the fact that the important reactions take place in the cell where they are especially effective for bringing about genetic changes.

The hypothesis that the reactions initiated by NMU are alkylations

is supported by some physiological observations. KIHLMAN (1960) found that the effect of NMU on root tip chromosomes of *Vicia faba* was dependent on temperature but independent of oxygen concentration. The radiomimetic effects of other alkylating agents, such as nitrogen mustard and di-(2,3-epoxypropyl)-ether, are also dependent on temperature but independent of oxygen concentration (KIHLMAN, 1955, 1956).

According to ALEXANDER *et al.* (1957) the alkylating agents are such effective mutagens because they combine with the most vulnerable groups in deoxyribonucleic acid (DNA), the phosphate groups. There is also an extensive combination with phosphate groups of DNA in reactions with nucleoproteins. Apparently alkylating agents can be very potent modifiers of the functioning genes.

WITKIN (1956) found that "ultraviolet-induced mutations are irreversibly established only after an appreciable delay". A delayed effect of treatment with alkylating agents has also been observed (AUERBACH, 1951), but as far as known it has not been experimentally tested if there is any correlation between the mechanisms of the observed delays. HAAS and DOUDNEY (1957, 1959 a, 1959 b, 1960) and DOUDNEY and HAAS (1958, 1959, 1960) have presented evidence that the mutagenic effects of UV are due to modifications of precursors of ribonucleic acid (RNA). Changes in RNA specificity might result in corresponding changes in gene DNA, and the manifestation of these changes is dependent on the synthesis of gene material in the cell. FORD (1949) and REVELL (1953) have shown that the chromosomes in root tip cells of *Vicia faba* are most sensible to alkylating agents (nitrogen mustard and diepoxypropylether respectively) during the early phase of resting stage, before chromosome duplication occurs. As shown by ALEXANDER *et al.* (1957) alkylation of RNA occurs readily by treatment with mutagenic alkylating agents. Thus a part of their mutagenic effects might also occur in this indirect way, although it is hard to explain the specific action found for some of them if they are supposed to act on gene precursors.

It has not been investigated here whether the mutations obtained with NMU are chromosome aberrations or gene mutations. However, seven of the mutants reverted to wild type during the process of identification. As far as known established chromosome aberrations are not reversible, and it is believed that NMU can induce gene or point mutations in *Ophiostoma.*

The NMU-treatment on *Ophiostoma* brought about a specific mutagenic effect, expressed as a different mutant spectrum and induction of

new biochemical mutant types. As soon as it was discovered that irradiation could induce mutations the important question arose, whether the mutations occurred randomly or selectively. For a long time the results reported gave support to the first alternative. In *Drosophila* the most common spontaneous mutations were also the most frequent after irradiation with X-rays (MULLER, 1952). It seemed as the irradiation resulted in an evenly distributed agitation of the genes, and that the most labile genes, with a high spontaneous mutation frequency, were most likely to mutate. However, as early as 1941 STADLER reported that there was a difference in the mutagenic action of UV and X-rays, the latter giving only nonreversible mutations. As shown later (DE SERRES, 1958; ZETTERBERG and FRIES, 1958) this statement did not hold for other material, but they were the first experiments to support further attempts to direct the mutation processes. When the mutagenic effects of chemicals were discovered the most fundamental question was if they were different from the mutagenic effects of radiation. In 1948 GUSTAFSSON and MAC KEY reported that nitrogen mustard-treatment of seeds of barley induced a higher proportion of *viridis* mutants than after X-radiation. In *Drosophila,* however, the results of the treatments with chemicals did not at first provide unequivocal evidence against the randomness of mutation, although some differences in the mode of action of X-rays and alkylating agents were reported (AUERBACH, 1951). In 1956 FAHMY and FAHMY made an extensive investigation of the effects of X-rays and alkylating compounds in *Drosophila* and reported differences regarding the frequency of visible mutations and the distribution of chromosome segments affected. FAVRET (1960) found that X-radiation induced mutations in four genes for albinism in barley. Treatments with ethyl methan-sulphonate were very mutagenic but induced mutations in only one of the four genes. Differences in the cytological effects of alkylating compounds and X-rays on plant material had earlier been reported by FORD (1949) and LOVELESS and REVELL (1949). They found that chromosome breaks induced by alkylating agents were most common in heterochromatic parts of the chromosomes in root tips of *Vicia.* Preferential chromosome breakage in *Vicia* after treatment with NMU was reported by KIHLMAN (1960). The breaks produced by NMU were localized preferably in the heterochromatic parts of the chromosomes.

Induction of biochemical mutants in microorganisms became a new and extremely useful approach to the understanding of mutation processes. Any specific effect of a mutagen could be expected to give a

different mutant spectrum. But such results did not appear at first. In 1950 TATUM *et al.* reported that the treatment with nitrogen mustard on *Neurospora* was "no more specific for the type of biochemical mutants produced than are X-ray and ultraviolet treatments". However, by the back-mutation test (GILES and LEDERBERG, 1948; KÖLMARK and WESTERGAARD, 1949) it was shown that some mutagenic treatments differed in their ability to induce back-mutations of the genes tested (KÖLMARK, 1953; GLOVER, 1956; ZETTERBERG, 1960 a). KAUDEWITZ (1959) obtained a different mutant spectrum in *Escherichia coli* and *Salmonella* by treatments with nitrous acid. BENZER and FREESE (1958) reported that 5-bromouracil induced specific mutations in phage. They were able to map these mutations on a molecular level and found that certain "hot spots" within the genes were most likely to be affected by the mutagen. Apparently there is good evidence available to support the conclusion that certain mutagenic treatments have affinity for special parts of the chromosomes or special genes or even special parts of the genes.

The high proportion of amino acid-less mutants in *Ophiostoma* after treatment with NMU is hard to explain. There is no evidence for a specific localization of the genes taking part in the synthesis of amino acids, and it seems unlikely that such genes should have a special chemical constitution in common which would make them especially exposed to mutagenic reactions initiated by NMU. So many assumptions have to be made that any further discussion at present seems unprofitable.

A comparison between *Ophiostoma* and *Neurospora* regarding different kinds of amino acid-less mutants (FRIES, 1948 a) shows that in *Neurospora* there are many more types of such mutants than in *Ophiostoma.* The fact that the mutagens earlier tried in *Ophiostoma* have given this narrow spectrum of amino acid-less mutants is perhaps more remarkable than the observation that NMU produced mutant types, well-known from other organisms, but never isolated in *Ophiostoma.*

Acknowledgement. — For valuable criticism and discussions I am greatly indebted to Professor NILS FRIES, who permitted me to publish some of his data, and to Dr. B. A. KIHLMAN, who suggested these experiments. Many thanks are also due to Mrs. EVA OLANDER for skilful technical assistance.

SUMMARY

N-nitroso-N-methylurethan (NMU) is a very potent mutagen in *Ophiostoma.* In two experiments the NMU-treatment has induced 3 and 4 per

cent mutants at a survival of 0.85 and 0.6 per cent respectively. A specific mutagenic effect was obtained with NMU, expressed as a different mutant spectrum and induction of new biochemical mutant types. The mode of action is discussed.

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PROPHASE BOUQUETS IN THE FIRST MEIOTIC DIVISION STUDIED IN BULLS WITH AND WITHOUT GENERAL ANAESTHESIA

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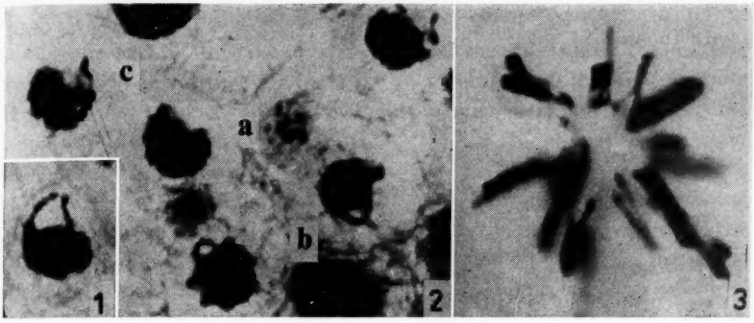
MELANDER and KNUDSEN (1953), among others, described a bouquet formation of the chromosomes in prophase of the first meiotic division of the bull. According to their observations, one, and only one, end of the bivalents is drawn to a single point at the nuclear periphery. Bouquets have also been interpreted as being made up of both ends of each chromosome lying at the nuclear membrane so that each bivalent forms a loop (LEUCHTENBERGER, SCHRADER, HUGHES-SCHRADER and GREGORY, 1956). These authors call for closed study of this stage of cell division. The additional material now at my disposal can resolve these conflicting views.

METHODS

The testicles of 10 fertile bulls of the Swedish Red and White breed aged between 1 and 7 years were obtained at slaughter or by castration in the manner previously described (KNUDSEN, 1954). For castration xylocaine was introduced into the funiculus spermaticus. Three of these bulls were castrated on one side and then immediately anaesthetized with Pentobarbital Sodium intravenously. After full anaesthesia for 5, 25, or 45 minutes (bulls A, B, and C, respectively), the other testicle was removed without a local block.

Small blocks of testicular tissue were fixed in acetic alcohol (1:3) within two minutes after shooting or the commencement of castration, being subsequently stained with Gomori's haematoxylin (MELANDER and WINGSTRAND, 1953) or by Feulgen's method (ROMEIS, 1948). In the histological sections prepared in this way chromosomes can be en-



Figs. 1—3. Meiotic prophase boquets. — Fig. 1. Two chromosomes attached end-to-end forming a "loop" outside the bouquet. Fig. 2. a: normal prophase bouquet; b: two nuclei, each with a chromosome loop, probably formed by a single chromosome; c: nucleus with a chromosome loop, probably formed by two or more chromosomes. Fig. 3. Diakinesis bouquet, centromeric ends of the chromosomes separated as far as the most proximal chiasma permits.

countered which extend beyond the open end of the pachytene bouquet, often forming a loop there (Figs. 1 and 2). In the sections from the bulls kept under general anaesthesia, similar "loops", consisting of a single bent chromosome or two or more chromosomes attached together, were studied in bouquets with their axis aligned approximately at right angles to the direction of light through the microscope. The relative numbers of these "loops" in testicles removed before and during general anaesthesia were counted by a person who was unaware of the origin of the sections.

RESULTS

The chromosomes are arranged as a bouquet from zygotene to diakinesis (see Fig. 6 in KNUDSEN and BRYNE, 1960). I have seen no reason to depart from the original description of bouquets in the 1953 paper (MELANDER and KNUDSEN); only one end of each bivalent is drawn towards the nuclear membrane. In diakinesis, the centrosomes are supposed to start a precocious separation. In this stage bivalents which lack a proximal chiasma form a loop between two points on the nuclear membrane produced by the centromeral end of the two homologous chromosomes of the bivalent having been drawn after the precociously separating centrosomes (Fig. 3). Details can be found in previous publications (MELANDER and KNUDSEN, 1953; KNUDSEN, 1954; KNUDSEN and BRYNE, 1960).

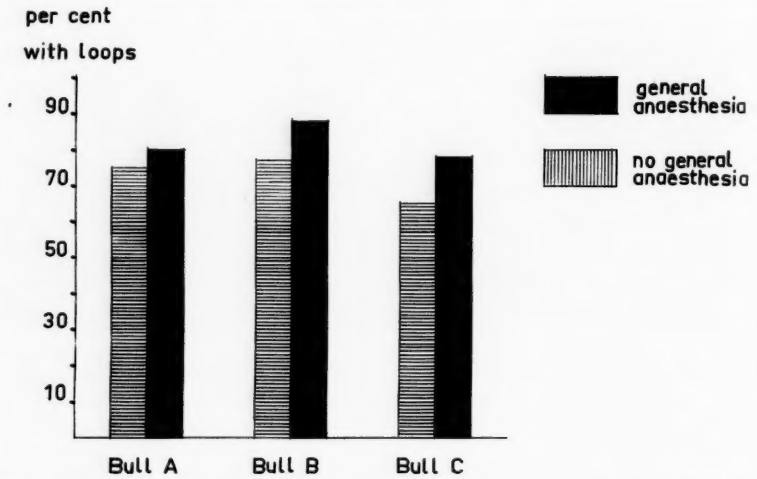


Fig. 4. Histogram showing prophase nuclei with chromosome loops outside the bouquet; bull A 5 minutes, bull B 25 minutes, and bull C 45 minutes under general anaesthesia.

The few chromosomes extending beyond the bouquet do not, for some reason or other, participate in polarisation. Such bivalents may extend straight out from the bouquet, form a simple loop (Fig. 2 b), or become attached end-to-end with another or several other bivalents to form a more discursive loop (Figs. 1 and 2 c). The number of cells containing such "loops" is significantly greater in testicles removed under general anaesthesia than under block anaesthesia (Table 1 and Fig. 4). For the individual bulls, the increase is significant on the ** level for bull B ($\chi^2=7.6$, 1 d.f. $0.01>P>0.001$), 25 minutes under anaesthesia, and bull C ($\chi^2=9.0$, 1 d.f. $0.01>P>0.001$), 45 minutes. An increase is apparent also for bull A, 5 minutes anaesthesia, although it is not significant.

TABLE 1. *The incidence of "chromosomal loops" beyond the bouquet of the prophase of the first meiotic division in bull testicles surgically removed with or without general (Pentobarbital Sodium) anaesthesia.*

	No. with "loops"	Percentage with "loops"	No. counted
General anaesthesia	491	81.83	600
No general anaesthesia	433	72.17	600
Total	924	77.00	1200

$\chi^2=15.82$ *** $P<0.001$

DISCUSSION

Only one end of the bivalents is gathered together along the nuclear membrane to form the bouquet in the prophase of the first meiotic division of the bull. Chromosome "looping", however, does occur at this stage and takes one of two patterns.

Premature separation of the centrosomes during diakinesis separates the branches of the bivalent as far as the position of the most proximal chiasma permits (Fig. 3), and in this manner forms a loop with the centromeral end of the chromosomes placed close to the nuclear membrane. Although this must be a normal appearance, also encountered by LEUCHTENBERGER *et al.*, it does not represent the gathering of both ends of the bivalents at the nuclear membrane since only the centromeral end of the chromosomes approaches the membrane.

The other type of "loop" is formed by chromosomes which have, for some reason, become detached from the bouquet and draped over the free ends of the bouquet. Since chromosomes, which extend straight out from the bouquet, may, in some cases, have a normally orientated centromeral end, only chromosomes forming loops were counted. The fact that the number of this latter type of "loop" increases highly significantly after one testicle has been removed under a regional block and the bull anaesthetized indicates that a few bouquet chromosomes are desorientated during the treatment. Thus, this type of "loop" must be considered to be an artefact and is probably not present at all in normal germinal epithelium *in vivo*.

When LEUCHTENBERGER *et al.* speak of "chromosome threads between the two centres some distance away at the nuclear membrane", they must refer to the bivalents of diakinesis, since premature separation of the centrosomes does not occur prior to this stage. The looped chromosomes which they observed in earlier stages may very well be the type seen particularly often in testicular tissue taken under general anaesthesia. Since they used nembutal (Pentobarbital Sodium) anaesthesia for castration of the bulls, the possibility that the looped chromosomes are an artefact merits careful consideration.

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SUMMARY

Chromosomal bouquet formation in the prophase of the first meiotic division in bulls has been re-investigated. Only one end of each bi-

valent is gathered together at a point on the nuclear membrane. Loops, however, can be formed by chromosomes detached from the bouquet. Since this type of loop is highly significantly more common in testicles taken under general (Pentobarbital Sodium) anaesthesia, it is presumed to be an artefact.

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ON MORPHOLOGICAL DIFFERENCES AND EVOLUTION OF DIFFERENT POLYPLOID PARTHENOGENETIC WEEVIL POPULATIONS

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I. INTRODUCTION

IN the weevils (Curculionidae) parthenogenesis is a very common phenomenon in the subfamilies Otiorrhynchinae and Brachyderinae. Although in those weevil genera in which parthenogenesis is to be found, there are closely related bisexual and parthenogenetic forms, parthenogenesis in the latter is always obligatory and thelytok. In addition, it is typical of the parthenogenesis of weevils that only one maturation division takes place in the eggs of the parthenogenetic forms, and this division is an equational one, so that there is no chromosome conjugation and no chromosome reduction (SUOMALAINEN, 1940 a and b; SEILER, 1947).

Another characteristic feature of parthenogenetic weevils is the common occurrence of polyploidy among them. In investigations on the chromosomal conditions of the parthenogenetic weevils (SUOMALAINEN, 1940 a and b, 1945, 1947, 1948, 1953, 1954, 1955; SEILER, 1947; MIKULSKA, 1949, 1951, 1953; TAKENOUCHI, 1957) it has been found that the majority of them are polyploid. Of the 34 parthenogenetic weevil species and races studied cytologically, only one (*Polydrosus mollis* in Finland and Poland) is diploid (22 chromosomes), 23 are triploid (with a chromosome number of 33 or thereabouts), 8 are tetraploid (44 chromosomes) and 2 (*Otiorrhynchus anthracinus* in Switzerland and *Barynotus moerens* in the Austrian Alps) are even pentaploid (55 chromosomes).

It is also to be noted that the coexistence of many different races is typical of many weevil species (SUOMALAINEN, op. cit.). Thus, a diploid

Dedicated to Professor J. SEILER on the occasion of his 75th birthday.

bisexual race and a polyploid parthenogenetic race are known in many species. It is to be noted that in many species a number of parthenogenetic races of different degrees of polyploidy are known besides the diploid bisexual race. The various races of a single weevil species have different distributions in all the cases so far investigated. This is especially clear, for instance, in some of the boreo-alpine species. In the Eastern Alps this dissimilar distribution of the divergent weevil races appears to be definitely correlated with the various glacial conditions in different parts of the Alps (JAHN, 1941).

As regards the origin of new polyploid forms, polyploidy itself plays an important rôle in the evolution of the parthenogenetic weevils. This is indicated, for instance, by the differences in distribution of divergent weevil races. For it is evident that these differences are primarily due to changes, produced by polyploidy, in the reaction norm and the optimum life conditions of these forms (cf. SUOMALAINEN, op. cit.).

As yet there are no investigations concerning the problem of whether the polyploid parthenogenetic weevil forms are capable of further evolution. On theoretical grounds it has been claimed (see e.g. WHITE, 1945, pp. 293—294; LÜERS and ULRICH, 1954, p. 600) that forms like the polyploid parthenogenetic weevils cannot evolve further. Evolution is assumed to have come to a standstill in these forms and they are thus thought to represent the end of that branch of phylogeny. To settle this point, an attempt has been made to compare the individuals of different populations of polyploid parthenogenetic weevils in order to establish whether populations like these remain unchanged or can differentiate.

II. MATERIAL

The material comprises only those parthenogenetic weevil species which have previously been studied cytologically by the author and whose chromosome number is therefore known (SUOMALAINEN, op. cit.). Similarly, populations which have actually been studied cytologically have primarily been chosen as objects of study. This ensures that the degree of polyploidy of the populations to be compared with each other in the following investigation is accurately known. As the ovaries of the weevils can be removed and the chromosome number determined from the ripe ova with very little, if any, breakage of the chitinous parts of the specimen, the chromosome number of many of the specimens measured in this investigation has been determined earlier.

The following weevil species have been included in the comparison:

Otiorrhynchus chrysocomus GM., *O. salicis* STRÖM, *O. scaber* L., *O. singularis* L. and *Peritelus hirticornis* HBST. These are the only species of which a sufficient amount of material has been preserved in connection with the cytological studies. Fuller details of the populations studied and the numbers of specimens measured will be presented in connection with the results obtained for each species.

The bulk of the material consists of specimens collected by the author from Finland, Germany, Switzerland and Austria. A few specimens have been collected by other persons. The Finnish material has been augmented by specimens from the collections of the Entomological Museum, University of Helsinki.

III. METHODS

The weevil specimens were measured with a Leitz binocular stereomicroscope (oculars 18 ×, objective 1 ×). A 1 cm. scale was attached to the ocular; this was subdivided into tenths and hundredths. Thus, one narrow subdivision (one hundredth of the scale) corresponds to 0.1 mm. All measurements were performed so that one end of the body part to be measured was placed precisely on the zero point of the scale, and the reading at the other end was recorded. In the readings the integral numbers refer to the broader divisions of the scale (i.e. millimeters) and the decimals to the narrower divisions (i.e. tenths of millimeters). If the end of the body part fell approximately at the middle of a narrower division, a second decimal was employed (this was always 5, for instance 2.35 instead of 2.3 or 2.4). Each specimen was provided with a number card to permit of a possible check of the measurements.

The following measurements were made on each specimen: the entire length of the animal, the length of head and thorax combined, maximum breadth, maximum breadth of the thorax, maximum height, and the length of both hind tibiae. The total length refers to the distance between the distal end of the beak and the distal end of the chitinous elytrae. The maximum breadth refers to the breadth of the broadest part of the specimen, which is almost without exception in the abdomen, near the junction of thorax and abdomen. The maximum height could not be determined from individuals whose ovaries had been removed for cytological examination, because the abdomen of these specimens had been opened on the ventral surface and the chitinous parts had often been wholly removed from this surface. When measuring the length of the hind tibiae, the animal was placed so that the tibia lay perpendicular to the plane of vision, and then the true length

of the tibia could be determined. If this was not possible, no measurement was taken. If there was a difference in length between the hind tibiae of the same specimen, the average length was used in the statistical analysis. In most cases there was little or no difference. (For instance, of those 114 Fennoscandian *Otiorrhynchus scaber* specimens whose hind tibiae were both measured, 70 per cent showed no difference in length between the tibiae, in 29 per cent the difference was half of a narrower division, and in 1 per cent — i.e. in one individual only — the difference was equal to one narrower division.)

IV. RESULTS OF MEASUREMENTS

The populations of the weevil species studied were compared with each other by calculating the ratio between total length and each of the other measurements successively (the length of head and thorax combined, maximum breadth, maximum breadth of the thorax, maximum height, and length of the hind tibiae).

In the following the results of the statistical analyses will be presented for each species separately.

1. Otiorrhynchus scaber L.

18 populations of this species were investigated; the origin, degree of polyploidy and number of specimens measured appear from the following tabulation.

	Number of specimens measured	Date captured [1]	Degree of polyploidy
Finland			
Espoo	8	1937	4n
Helsinki	30		4n
Lohja	13		4n
Parainen	7		4n
Pohja	6	1938—39	4n
Porvoo	20	1943, 1953—54	4n
Tampere	7		4n
USSR			
Kivennapa (Karelian Isthmus)	7		4n ?
Kuolemajärvi (Karelian Isthmus)	16	1920—29	4n ?
Kuujärvi (Olonetz)	5	1943	4n ?
Norway			
Trondheim, Bymark	4	1947	4n ?

[1] The year is not mentioned where the material was collected in the course of several years or where the year of capture is not known.

Switzerland			
Ortschwaben (Canton Bern)	15	1948	3n
Langnau (Emmental)	13	1948	3n; 4n
Gurnigel (Canton Bern)	13	1948, 1957	4n
Zeneggen (Oberwallis)	12	1948	4n
Zürich, Zürichberg	21	1948	4n
Austria			
Lunz am See (Niederdonau)	46	1943	3n
Innerkrems (Ober-Kärnten)	21	1954	? (3n or 4n)

The means for different *Otiorrhynchus scaber* populations appear from Table 1.

TABLE 1. *The means for the Otiorrhynchus scaber populations investigated.*

	Length	Length of head and thorax combined	Breadth	Breadth of the thorax	Height	Hind tibiae	
						left	right
Innerkrems	5.65	2.25	2.61	1.53	2.18	1.51	1.47
Lunz am See	5.33	2.01	2.33	1.40	1.89	1.44	1.44
Zeneggen	5.74	2.29	2.65	1.52	2.20	1.56	1.57
Langnau	5.59	2.25	2.53	1.55	2.04	1.59	1.58
Ortschwaben	5.59	2.14	2.42	1.53	2.02	1.58	1.58
Gurnigel	5.74	2.29	2.65	1.51	2.15	1.59	1.60
Zürich	5.83	2.35	2.62	1.56	2.16	1.64	1.63
Trondheim	5.78	2.33	2.58	1.54	2.18	1.69	1.68
Parainen	5.59	2.10	2.62	1.59	2.29	1.64	1.62
Pohja	5.49	2.11	2.57	1.54	2.23	1.57	1.57
Lohja	5.40	2.18	2.58	1.58	2.25	1.62	1.61
Espoo	5.73	2.35	2.62	1.60	2.30	1.68	1.67
Helsinki	5.65	2.23	2.65	1.57	2.27	1.62	1.63
Porvoo	5.84	2.30	2.64	1.57	2.28	1.60	1.60
Tampere	5.72	2.36	2.68	1.60	2.35	1.65	1.66
Kuolemajärvi	5.69	2.13	2.61	1.59	2.35	1.65	1.66
Kivennapa	5.52	2.18	2.63	1.56	2.25	1.59	1.58
Kuujärvi	5.95	2.23	2.71	1.61	2.34	1.65	1.67

When we compare the mean total lengths of different populations, it appears that this value is smaller in triploid (Lunz am See, Ortschwaben) and mixed triploid-tetraploid populations (Langnau) than in most tetraploid populations. The specimens in Lunz am See are on average the smallest ones. The mean total length in the triploid populations investigated is 5.46, whereas it is 5.69 in the tetraploid populations ($t=5.3$, d.f.$=224$, $P<0.001$). This means that the specimens in tetra-

TABLE 2. *Analysis of variance of the ratio total length:length of head and thorax combined in the Otiorrhynchus scaber populations investigated.*

	Innerkrems	Lunz am See	Zeneggen	Langnau	Ortschwaben	Gurnigel	Zürich	Trondheim	Parainen	Pohja	Lohja	Espoo	Helsinki	Porvoo	Tampere	Kuolemajärvi	Kivennapa	Kuujärvi
n	21	46	12	13	15	13	21	4	5	6	12	8	29	20	7	16	7	5
$\bar{X}$	2.52	2.65	2.49	2.48	2.61	2.52	2.48	2.49	2.70	2.63	2.49	2.45	2.54	2.55	2.43	2.68	2.56	2.67

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	259	2566			
Between populations	17	561.2	33.01	4.0	0.001
Error	242	2005	8.29		

TABLE 3. *Analysis of variance of the ratio total length:maximum breadth in the Otiorrhynchus scaber populations investigated.*

	Innerkrems	Lunz am See	Zeneggen	Langnau	Ortschwaben	Gurnigel	Zürich	Trondheim	Parainen	Pohja	Lohja	Espoo	Helsinki	Porvoo	Tampere	Kuolemajärvi	Kivennapa	Kuujärvi
n	21	46	12	13	15	12	21	4	5	6	12	8	29	20	7	16	7	5
$\bar{X}$	2.16	2.30	2.16	2.23	2.31	2.18	2.22	2.24	2.12	2.14	2.08	2.19	2.13	2.21	2.13	2.10	2.11	2.20

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	257	1005			
Between populations	17	518.3	30.49	14.9	0.001
Error	240	486.7	2.03		

TABLE 4. *Analysis of variance of the ratio total length : maximum breadth of the thorax in the Otiorrhynchus scaber populations investigated.*

	Innerkrems	Lunz am See	Zeneggen	Langnau	Ortschwaben	Gurnigel	Zürich	Trondheim	Parainen	Pohja	Lohja	Espoo	Helsinki	Porvoo	Tampere	Kuolemajärvi	Kivennapa	Kuujärvi
n	21	46	12	13	15	13	21	4	7	6	12	8	29	20	7	16	7	5
$\bar{X}$	3.69	3.77	3.68	3.61	3.67	3.80	3.74	3.76	3.51	3.53	3.41	3.58	3.59	3.74	3.58	3.57	3.53	3.70

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	261	2385			
Between populations	17	1090	64.1	12.1	0.001
Error	244	1295	5.31		

TABLE 5. *Analysis of variance of the ratio total length : length of hind tibiae in the Otiorrhynchus scaber populations investigated.*

	Innerkrems	Lunz am See	Zeneggen	Langnau	Ortschwaben	Gurnigel	Zürich	Trondheim	Parainen	Pohja	Lohja	Espoo	Helsinki	Porvoo	Tampere	Kuolemajärvi	Kivennapa	Kuujärvi
n	17	36	11	13	15	13	21	4	5	6	11	8	26	20	7	16	6	5
$\bar{X}$	3.81	3.75	3.73	3.56	3.54	3.60	3.59	3.42	3.43	3.51	3.34	3.43	3.49	3.66	3.45	3.44	3.49	3.57

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	239	3539			
Between populations	17	1642	96.59	11.3	0.001
Error	222	1897	8.55		

ploid *O. scaber* populations are, on the average, longer than triploid specimens, although the difference is not great.

The same holds true with regard to the maximum breadth of triploid and tetraploid specimens. The average breadth of both the triploid populations as well as of the mixed triploid and tetraploid population is smaller than the average breadth in any tetraploid population. The mean maximum breadth is 2.38 in triploid and 2.63 in tetraploid populations ($t=10.3$, d.f.$=210$, $P<0.001$).

Tetraploid Otiorrhynchus scaber specimens are thus clearly bigger than triploid ones, although the difference is not great.

A comparison between all *O. scaber* populations was undertaken in the manner described on page 312. The analysis of variance is seen from Tables 2—5. In this case, the ratio total length:maximum height was not calculated, as the ovaries had been removed from many specimens for cytological examination.

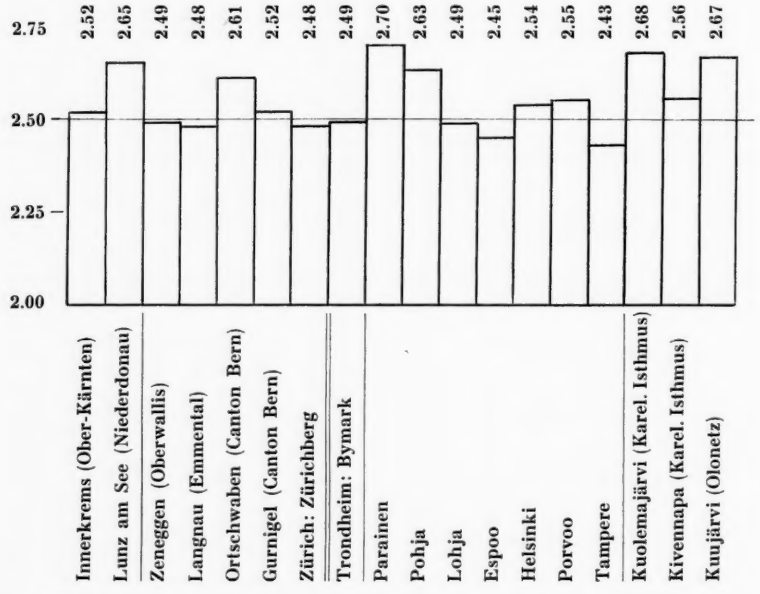

Fig. 1. The ratio of total length to length of head and thorax combined in the *Otiorrhynchus scaber* populations investigated. The values of the ordinate refer to the ratio mentioned.

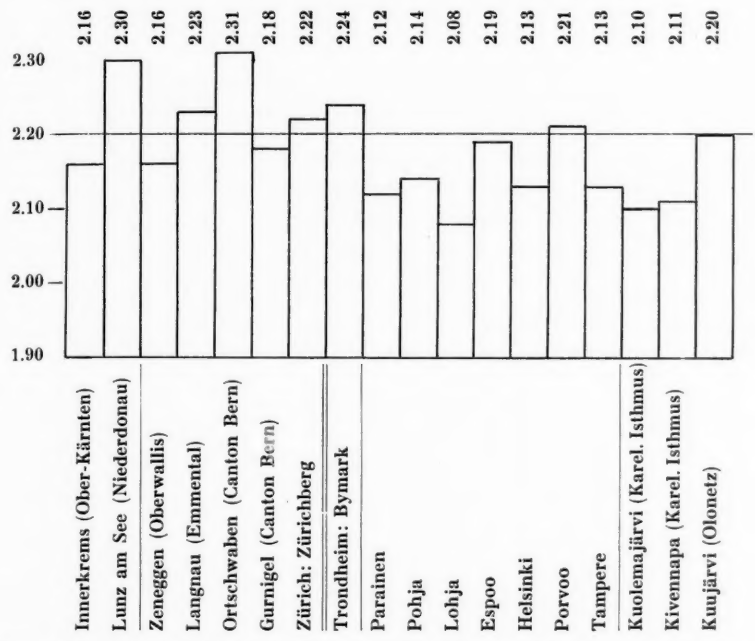

Fig. 2. The ratio of total length to maximum breadth in the *Otiorrhynchus scaber* populations investigated. The values of the ordinate refer to the ratio mentioned.

The results of the statistical analysis. — The analysis of variance shows clearly that the *Otiorrhynchus scaber* populations investigated can be divided into two groups. The one comprises most Central European populations (Swiss and Austrian populations) whereas the other comprises most Fennoscandian populations. (See Figs. 1—4, in which the results of the statistical analysis are presented graphically.) This division into two groups holds true with regard to every ratio with the exception of the ratio total length : length of head and thorax combined (Table 2, Fig. 1).

When we examine the ratio total length : maximum breadth, it emerges that (Table 3, Fig. 2) the specimens of the triploid populations (Lunz am See, Ortschwaben) are decidedly the narrowest. Further, the other Austrian and Swiss populations as well as the Norwegian population are relatively narrower than most of the Finnish and Karelian populations. Of the latter, the specimens collected from Porvoo, Kuujärvi and Espoo are narrower than the others.

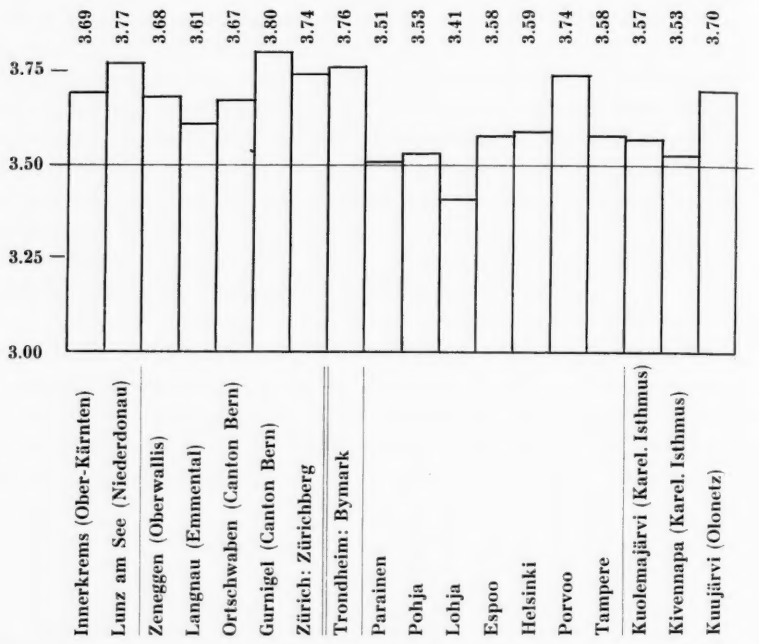

Fig. 3. The ratio of total length to maximum breadth of the thorax in the *Otiorrhynchus scaber* populations investigated. The values of the ordinate refer to the ratio mentioned.

The picture given by the ratio total length:maximum breadth of the thorax is very similar to that shown by the ratios mentioned above (Table 4, Fig. 3). The populations of Central Europe (with the exception of the population in Langnau) and the Norwegian population have on the average a distinctly narrower thorax than Finnish and Karelian specimens. From the latter, the populations of Porvoo and Kuujärvi deviate clearly: they have a thorax relatively as narrow as the Central European populations.

As regards the relative length of the hind tibiae (Table 5, Fig. 4), the specimens of the Central European populations uniformly possess relatively shorter tibiae than the Fennoscandian populations. In this respect the Norwegian population resembles other Fennoscandian populations. The populations of Porvoo and Kuujärvi are clearly exceptional among the Fennoscandian populations. It is especially interesting that the Por-

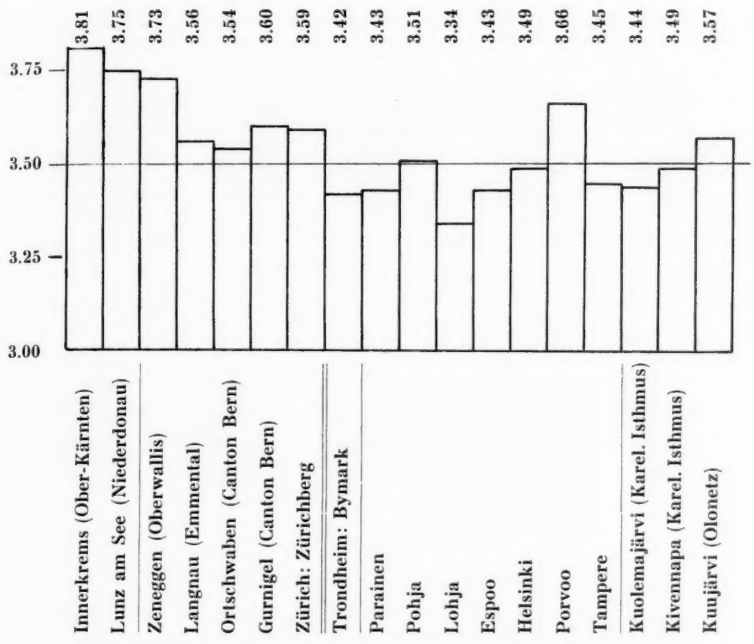

Fig. 4. The ratio of total length to length of hind tibiae in the *Otiorrhynchus scaber* populations investigated. The values of the ordinate refer to the ratio mentioned.

voo population deviates so strikingly from the other Fennoscandian populations, the tibiae being relatively even shorter than in the majority (four out of seven) of Central European populations.

As appears from the analysis of variance, the differences reviewed above are statistically significant.

It is to be noted that the population of Lunz am See (Austria) differs from the other populations even *in coloration*. The specimens of this population are more greyish brown and there is no embellishment on their elytrae, in contrast to the other populations (Fig. 5). That the specimens collected in Lunz am See really belong to this species has been confirmed by Prof. Dr. K. HOLDHAUS (Vienna).

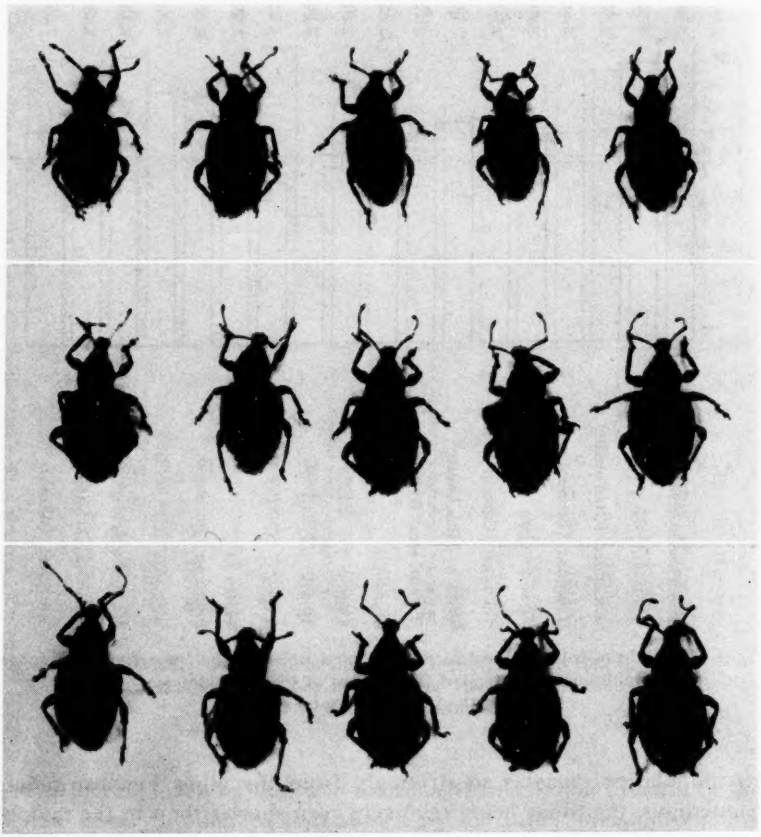

Fig. 5. Specimens of *Otiorrhynchus scaber* examined. Top row: Lunz am See, Austria. Triploid individuals. Middle row: Porvoo, Finland. Tetraploid individuals. Bottom row: Zeneggen, Switzerland. Tetraploid individuals. 4 ×.

2. Otiorrhynchus chrysocomus Gm.

The data on this species refer to two populations, both from Oberwallis in Switzerland. These are the population of Zeneggen (27 specimens) and that of Berisal (16 specimens). These two populations inhabit different valleys separated by a distance of about 16 km. Both populations are triploid.

The means for the two populations are given in Table 6.

TABLE 6. *The means for the Otiorrhynchus chrysocomus populations investigated.*

	Length	Length of head and thorax combined	Breadth	Breadth of the thorax	Height	Hind tibiae left	Hind tibiae right
Zeneggen	8.86	3.57	3.84	2.33	3.19	2.26	2.26
Berisal	8.97	3.56	3.85	2.34	3.13	2.17	2.17

The comparison between the populations was performed in the manner described on page 312. The results of the statistical analysis are seen in Tables 7—11.

TABLE 7. *Analysis of variance of the ratio total length:length of head and thorax combined in the Otiorrhynchus chrysocomus populations investigated.*

	Zeneggen	Berisal
n	27	16
$\bar{X}$	2.51	2.52

F	P<
—	—

TABLE 8. *Analysis of variance of the ratio total length:maximum breadth in the Otiorrhynchus chrysocomus populations investigated.*

	Zeneggen	Berisal
n	27	16
$\bar{X}$	2.34	2.32

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	42	31.1			
Between populations	1	1.7	1.7	2.4	—
Error	41	29.4	0.72		

TABLE 9. *Analysis of variance of the ratio total length:maximum breadth of the thorax in the Otiorrhynchus chrysocomus populations investigated.*

	Zeneggen	Berisal
n	27	16
$\bar{X}$	3.84	3.79

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	42	268.3			
Between populations	1	8.7	8.70	1.37	0.25
Error	41	259.6	6.33		

TABLE 10. *Analysis of variance of the ratio total length : maximum height in the Otiorrhynchus chrysocomus populations investigated.*

		Zeneggen		Berisal		
	n	12		4		
	$\overline{X}$	2.78		2.84		
Source of variation	D.f.	Sx^2	s^2		F	P<
Total	15	33.7				
Between populations	1	4.0	4.0		1.9	—
Error	14	29.7	2.12			

TABLE 11. *Analysis of variance of the ratio total length : length of hind tibiae in the Otiorrhynchus chrysocomus populations investigated.*

		Zeneggen		Berisal		
	n	27		16		
	$\overline{X}$	3.98		4.13		
Source of variation	D.f.	Sx^2	s^2		F	P<
Total	42	204.2				
Between populations	1	96.3	96.30		36.6	0.001
Error	41	107.9	2.63			

The results of the statistical analysis. — The relative length of the hind tibiae is clearly greater in the Zeneggen population than in that of Berisal. The difference is statistically significant. In the other ratios no differences are found between the two populations.

3. Otiorrhynchus salicis Ström

The data on this species have been obtained from four populations:

	Number of specimens measured	Date captured	Degree of polyploidy
Norway			
Ustaoset	9	1948	3n
Namdalen, Deråas	3	1947	?
Switzerland			
Berisal (Oberwallis)	34	1948	3n
Austria			
Lunz am See (Niederdonau)	22	1943	2n; 3n

The population in Lunz am See comprises both a diploid bisexual (males and females) and a triploid parthenogenetic race. At the time of collecting the material, it was not known that both these races occur there, and therefore they are treated together in the following.

The means for different populations of *Otiorrhynchus salicis* appear from Table 12.

TABLE 12. *The means for the Otiorrhynchus salicis populations investigated.*

	Length	Length of head and thorax combined	Breadth	Breadth of the thorax	Height	Hind tibiae left	Hind tibiae right
Ustaoset	7.58	2.85	3.19	1.83	2.84	2.08	2.08
Namdalen	8.60	3.25	3.58	2.13	3.35	2.33	2.33
Berisal	8.05	3.09	3.30	1.94	3.02	2.17	2.17
Lunz am See	7.70	3.09	2.98	1.86	2.73	2.27	2.26

The Norwegian specimens from Namdalen are clearly bigger than the specimens of the other populations; however, it is true that the data comprise only 3 specimens. Whether the large size of these specimens is due to a higher degree of polyploidy is impossible to say at this stage, since the chromosome number of these specimens could not be determined.

The populations of *O. salicis* were compared with each other in the same way as those of other species. The results of the statistical analysis of the ratio of total length to all other measurements are shown in Tables 13—17.

TABLE 13. *Analysis of variance of the ratio total length:length of head and thorax combined in the Otiorrhynchus salicis populations investigated.*

	Ustaoset	Namdalen	Berisal	Lunz am See	
n	9	3	32	22	
$\bar{X}$	2.66	2.65	2.61	2.50	
Source of variation	D.f.	Sx^2	s^2		
Total	65	319.5		F	P<
Between populations	3	100.1	33.40	9.4	0.001
Error	62	219.4	3.54		

TABLE 14. *Analysis of variance of the ratio total length:maximum breadth in the Otiorrhynchus salicis populations investigated.*

	Ustaoset	Namdalen	Berisal	Lunz am See	
n	9	3	33	22	
$\bar{X}$	2.38	2.40	2.44	2.60	
Source of variation	D.f.	Sx^2	s^2		
Total	66	392.3		F	P<
Between populations	3	204.5	68.2	22.9	0.01
Error	63	187.8	2.98		

TABLE 15. *Analysis of variance of the ratio total length: maximum breadth of the thorax in the Otiorrhynchus salicis populations investigated.*

	Ustaoset	Namdalen	Berisal	Lunz am See	
n	9	3	34	22	
$\bar{X}$	4.11	4.03	4.16	4.15	
Source of variation	D.f.	Sx^2	s^2		
Total	67	229		F	P<
Between populations	3	24	8.0	2.50	0.1
Error	64	205	3.2		

TABLE 16. *Analysis of variance of the ratio total length: maximum height in the Otiorrhynchus salicis populations investigated.*

	Ustaoset	Namdalen	Berisal	Lunz am See	
n	5	—	13	21	
$\bar{X}$	2.75		2.65	2.82	
Source of variation	D.f.	Sx^2	s^2		
Total	38	435.9		F	P<
Between populations	2	89.2	44.6	4.6	0.02
Error	36	346.7	9.63		

TABLE 17. *Analysis of variance of the ratio total length: length of hind tibiae in the Otiorrhynchus salicis populations investigated.*

	Ustaoset	Namdalen	Berisal	Lunz am See	
n	9	3	34	22	
$\bar{X}$	3.66	3.68	3.72	3.40	
Source of variation	D.f.	Sx^2	s^2		
Total	67	673.1		F	P<
Between populations	3	538.3	179.4	85.0	0.001
Error	64	134.8	2.11		

The results of the statistical analysis. — The population of Lunz am See differs from the others in having a relatively greater length of head and thorax combined, in being relatively narrower and flatter and in having relatively longer hind tibiae than the other populations. All this, however, may be due to the fact that the material from Lunz am See also includes males.

Otherwise, the differences between the populations are very slight. It is to be noted that the Norwegian populations do not notably differ from the Swiss population, except that possibly the specimens from Namdalen are bigger than the rest.

4. Otiorrhynchus singularis L.

Seven populations of this species were investigated. The origin, degree of polyploidy and number of specimens measured are tabulated below.

	Number of specimens measured	Date captured	Degree of polyploidy
Finland			
Helsinki	18		3n
Germany			
Berlin-Buch	10	1943	3n
Switzerland			
Lägern (Canton Zürich)	11	1948	3n
Zürich: Waldeck	5	1948	3n
Zürich: Zürichberg	23	1948	3n
Signau (Emmental)	21	1948	3n
Mendrisio (Tessin) (*O. carmagnolae* Villa)	6	1948	2n

All populations except the last are triploid. The material also comprises 6 specimens of *O. carmagnolae* VILLA collected from Mendrisio in Switzerland (Canton Tessin), because it seems possible that this species is a bisexual race of *O. singularis* (see SUOMALAINEN, 1954, p. 648). The measured specimens of this population are thus diploid and the data also refer to males.

The means of measurements of different *O. singularis* populations are given in Table 18.

TABLE 18. *The means for the Otiorrhynchus singularis populations investigated.*

	Length	Length of head and thorax combined	Breadth	Breadth of the thorax	Height	Hind tibiae	
						left	right
Mendrisio (*O. carmagnolae*)	5.78	2.40	2.48	1.62	2.00	1.72	1.68
Signau	7.50	3.20	3.17	1.99	2.50	1.89	1.89
Zürich: Waldeck	7.70	3.16	3.21	2.04	2.52	1.92	1.92
Zürich: Zürichberg	7.44	2.92	3.17	1.93	2.35	1.83	1.83
Lägern	7.37	3.06	3.15	1.96	2.46	1.84	1.83
Berlin-Buch	7.29	3.06	3.16	1.98	2.53	1.92	1.90
Helsinki	7.19	3.08	3.10	1.93	2.56	1.85	1.84

The comparison was performed in the same manner as above. The results of statistical analysis of the ratio of total length to all other measurements are shown in Tables 19—23.

TABLE 19. *Analysis of variance of the ratio total length: length of head and thorax combined in the Otiorrhynchus singularis populations investigated.*

	Mendrisio	Signau	Zürich: Waldeck	Zürichberg	Lägern	Berlin-Buch	Helsinki
n	6	21	5	23	11	10	18
$\bar{X}$	2.43	2.35	2.44	2.54	2.41	2.40	2.35

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	93	677.2			
Between populations	6	209.5	34.92	6.27	0.001
Error	87	467.7	5.57		

TABLE 20. *Analysis of variance of the ratio total length: maximum breadth in the Otiorrhynchus singularis populations investigated.*

	Mendrisio	Signau	Zürich: Waldeck	Zürichberg	Lägern	Berlin-Buch	Helsinki
n	6	21	5	23	11	10	18
$\bar{X}$	2.33	2.36	2.40	2.33	2.34	2.31	2.33

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	93	194.3			
Between populations	6	18.5	3.08	1.47	0.2
Error	87	175.8	2.09		

TABLE 21. *Analysis of variance of the ratio total length: maximum breadth of the thorax in the Otiorrhynchus singularis populations investigated.*

	Mendrisio	Signau	Zürich: Waldeck	Zürichberg	Lägern	Berlin-Buch	Helsinki
n	6	21	5	22	11	10	18
$\bar{X}$	3.57	3.78	3.78	3.87	3.76	3.67	3.72

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	92	781.3			
Between populations	6	167.2	27.87	3.90	0.005
Error	86	614.1	7.14		

TABLE 22. *Analysis of variance of the total length: maximum height in the Otiorrhynchus singularis populations investigated.*

	Mendrisio	Signau	Zürich: Waldeck	Zürichberg	Lägern	Berlin-Buch	Helsinki
n	—	15	5	22	—	—	15
$\bar{X}$		2.98	3.06	3.18			2.79

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	56	793.0			
Between populations	3	552.9	184.30	40.7	0.001
Error	53	240.1	4.53		

TABLE 23. *Analysis of variance of the ratio total length:length of hind tibiae in the Otiorrhynchus singularis populations investigated.*

	Mendrisio	Signau	Zürich: Waldeck	Zürichberg	Lägern	Berlin-Buch	Helsinki
n	6	21	5	23	11	9	18
$\bar{X}$	3.44	3.96	4.02	4.08	4.01	3.86	3.90

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	92	1458.9			
Between populations	6	821.9	136.98	18.5	0.001
Error	86	637.0	7.41		

The results of the statistical analysis. — It appeared that there are statistically significant differences (with the exception of the ratio total length:maximum breadth) between populations of *Otiorrhynchus singularis*, although the differences are in most cases not great. The clearest deviation is shown by the population of Mendrisio, Tessin (*O. carmagnolae*), which differs from the other populations in having a relatively much broader thorax and considerably longer hind tibiae. This difference is readily understandable, since *O. carmagnolae* is bisexual and diploid and most of the specimens measured are males. The population of Zürichberg, too, differs distinctly from the others with regard to most of the traits examined. The length of head and thorax combined is clearly smaller, the thorax is narrower, the height is smaller and the tibiae shorter than in other populations. On the other hand, the populations of Berlin and Helsinki have relatively longer hind tibiae than the other populations (with the exception of *O. carmagnolae*), and the population of Helsinki is, in addition, remarkable in possessing the greatest average height. All these and other smaller differences can be seen from the tables.

5. Peritelus hirticornis Hbst.

The data on this species have been obtained from three populations:

Switzerland	Number of specimens measured	Date captured	Degree of polyploidy
Ortschwaben (Canton Bern)	7	1948	3n
Signau (Emmental)	5	1948	3n
Zeneggen (Oberwallis)	8	1948	4n

The means for the three populations are given in Table 24.

TABLE 24. *The means for the Peritelus hirticornis populations investigated.*

	Length	Length of head and thorax combined	Breadth	Breadth of the thorax	Height	Hind tibiae left	Hind tibiae right
Ortschwaben	5.90	2.30	2.16	1.35	1.65	1.43	1.44
Signau	6.05	2.37	2.27	1.36	1.72	1.39	1.41
Zeneggen	6.46	2.54	2.47	1.53	1.84	1.54	1.53

The tetraploid specimens of this species, too, are clearly bigger than the triploid ones, the mean length of the former being 6.53 and of the latter 5.98. (This comparison includes a tetraploid specimen collected in Sumiswald, Switzerland; it has, however, been omitted from the statistical analysis.)

The populations of *Peritelus hirticornis* were compared with each other in the same way as those of other species. The results of the statistical analysis are seen in Tables 25—28.

TABLE 25. *Analysis of variance of the ratio total length:length of head and thorax combined in the Peritelus hirticornis populations investigated.*

	Ortschwaben	Signau	Zeneggen		
n	7	5	8		
$\bar{X}$	2.56	2.54	2.54		
Source of variation	D.f.	Sx^2	s^2	F	P<
Total	19	36.95			
Between populations	2	1.25	0.63	<1	—
Error	17	35.7	2.1		

TABLE 26. *Analysis of variance of the ratio total length:maximum breadth in the Peritelus hirticornis populations investigated.*

	Ortschwaben	Signau	Zeneggen		
n	7	5	8		
$\bar{X}$	2.74	2.66	2.61		
Source of variation	D.f.	Sx^2	s^2	F	P<
Total	19	58.2			
Between populations	2	25.1	12.55	6.4	0.01
Error	17	33.1	1.95		

TABLE 27. *Analysis of variance of the ratio total length:maximum breadth of the thorax in the Peritelus hirticornis populations investigated.*

	Ortschwaben	Signau	Zeneggen
n	7	5	8
$\bar{X}$	4.37	4.44	4.22

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	19	146.9			
Between populations	2	68.5	34.25	7.5	0.001
Error	17	78.4	4.61		

TABLE 28. *Analysis of variance of the ratio total length:length of hind tibiae in the Peritelus hirticornis populations investigated.*

	Ortschwaben	Signau	Zeneggen
n	7	5	8
$\bar{X}$	4.11	4.30	4.23

Source of variation	D.f.	Sx^2	s^2	F	P<
Total	19	245			
Between populations	2	46.1	23.1	2.0	—
Error	17	198.9	11.7		

The results of the statistical analysis. — The specimens of the tetraploid population of Zeneggen clearly have a relatively broader thorax and the specimens are also a little broader than those of the triploid populations investigated (Ortschwaben and Signau). No significant differences could be detected with regard to the other traits investigated.

V. DISCUSSION

1. On the morphological differences between the weevil populations studied

The above analysis has shown that different populations of the same parthenogenetic weevil species having the same degree of polyploidy exhibit significant and in some cases great morphological differences. These differences may, of course, be due to different environmental conditions, but they may also have a genetic basis. It is naturally difficult to say, without experimental investigations, which of these two groups of factors have caused these differences. Some speculations, however, can be advanced here.

The material of *Otiorrhynchus scaber* is the amplest one in this investigation, with regard to the numbers of both populations and specimens. This species may thus be taken as an example in our theoretical considerations. With regard to most of the traits examined here, the populations of *O. scaber* can be divided into two groups. The first comprises most Central European ('Alpine') populations and the second most North European populations. This mere bipartition may naturally be due to the prevailing differences in environmental conditions between the areas inhabited by these populations. It is to be noted, however, that among the North European populations there are some (especially the population from Porvoo, on the south coast of Finland) which deviate remarkably from those of adjacent areas in which the environmental conditions are similar; at the same time these deviating populations closely resemble the Central European populations investigated. The situation is basically the same in some other weevil species studied. Differences of this kind can, to my mind, scarcely be attributed to different environmental conditions alone. Therefore, it seems very probable that *the differences between weevil populations detected in this investigation owe their presence, at least to a considerable degree, to differences in the gene pools of the populations.*

On the other hand, the genotypic differentiation of the weevil populations studied indicates that, contrary to the earlier assumptions made on theoretical grounds (see p. 310), *evaluation does not come to a complete standstill in polyploid parthenogenetic populations.* Polyploid parthenogenetic weevils and other similar forms still possess some mechanism which allows the genotypic differentiation of populations and thus secures continued evolution.

2. On the factors restraining the evolution of parthenogenetic weevils

When we analyse the evolutionary possibilities of the parthenogenetic weevils closely, we must especially keep an eye open for two unusual features which play a very important role in their evolution, namely the exceptional maturation division of their ova, and their polyploidy.

As already mentioned on page 309, only one maturation division takes place in the eggs of the parthenogenetic weevils. This division is an equational one, so that there is no chromosome conjugation and no chromosome reduction (SUOMALAINEN, 1940 a and b, 1947; SEILER, 1947). SEILER (op. cit.) has found that the eggs of the parthenogenetic

Otiorrhynchus sulcatus undergo "a rudimentary reduction division", in addition to an equational division, but even in this case, there is no chromosome conjugation or reduction. Thus, the new gene combinations which are formed in the normal course of meiosis and provide an important part of the material basis of evolution, cannot arise in the parthenogenetic weevils. This in itself limits the evolution of these species. Apart from mutations, the offspring retain precisely the same genetic constitution as the mother.

What has been said above does not apply to every kind of parthenogenesis, but only to such cases as the weevils, in which the ova undergo a single equational maturation division. These species thus display the apomictic or ameiotic type of parthenogenesis. In automictic, or meiotic, parthenogenesis meiosis is normal and consists of two maturation divisions, and chromosome conjugation, crossing over and chromosome reduction take place. Whether the heterozygosity of a pair of alleles is maintained in automictic parthenogenesis or not depends on the manner in which the unreduced chromosome number is restored. (For details, see SUOMALAINEN, 1940 c, pp. 25—28; 1950, pp. 217—223; SMITH, 1941, pp. 297—298, and WHITE, 1945, pp. 282—283.) In most such cases heterozygosity is inevitably replaced by homozygosity in the progeny. From this it follows that a recessive mutation has great possibilities to become homozygous and to be expressed in the phenotype almost immediately after it occurs. The possibilities for evolution are thus greater in automictic parthenogenesis than in the apomictic type occurring in the weevils.

Another factor which exerts a great influence on the evolution of the parthenogenetic weevils is their polyploidy. It was mentioned on page 309 that parthenogenetic weevils are for the most part polyploid, the degree of polyploidy being most frequently triploidy. Tetraploidy is also relatively common and in some cases the degree of polyploidy has even risen to pentaploidy. This brings us to the problem of evolution in polyploid forms.

Since there is no fertilization or chromosome reduction in parthenogenetic weevils, the rôle of hybridization and segregation in the evolution of polyploids need not concern us here and we shall confine our discussion to the mutations. There are no experiments or theoretical calculations of the frequency of mutations among the weevils. However, we can start from the assumption that in this respect the weevils are not exceptional among other organisms of corresponding kind. There is no reason to suppose that parthenogenesis would diminish the chance

of mutation. On the contrary, GENTCHEFF and GUSTAFSSON (1940), using *Hieracium robustum* among other objects of study, have shown that chromosomal rearrangements are common in apomicts. However, polyploids differ from diploid organisms in that it is very difficult for a mutation — and this concerns gene mutations explicitly — to affect the phenotype in them. For instance, if a gene mutation occurs in an autotetraploid form, three alleles remain unchanged and the new, generally recessive, gene does not become expressed. DOBZHANSKY (1937, p. 225), HUSKINS (1941, p. 334), WHITE (1945, p. 293) and GUSTAFSSON (1947 b, p. 187), among others, have stressed the evolutionary significance of this circumstance. This is not a mere theoretical consideration: in X-ray experiments STADLER (1929, 1932) found that the number of mutations which gained expression was, indeed, greater in diploid plants than in polyploids of the same genus which had been treated in a similar manner. If a mutation should eventually occur in one of the alleles of the mutated gene, it is very improbable that this new mutation would be similar to the former. Thus, it is unlikely that the alleles at the same locus would all mutate similarly, even if the frequency of gene mutations were high in the polyploid form in question; more than one dose of a gene, however, is generally a prerequisite for the expression of the mutation in the phenotype of polyploids.

We have already mentioned that meiosis, which always makes it possible for the genes in forms having normal meiosis to reach homozygosity and thus allows the expression of recessive mutations, too, does not lead to gene recombination in the parthenogenetic weevils. Theoretically, at least, the only gene mutations that can be expressed in the phenotype of the polyploid parthenogenetic weevils are those dominants which exert an effect strong enough to overshadow that of two or more alleles at the same locus. Such mutations are rare, however.

As was already mentioned on page 310, it has often been claimed for the above-mentioned reasons that forms like the polyploid parthenogenetic weevils are incapable of further evolution. Thus, it is believed that evolution has come to a standstill in these forms and that they represent a "blind alley" in evolution.

3. Evolutionary possibilities in apomictic plants

Since there is no chromosome reduction in apomictic plants either and since polyploidy is also a common phenomenon among them, they offer an interesting object of comparison in studies on the evolution of the parthenogenetic weevils.

It is typical of those plant genera in which apomixis is common that they comprise a great number of so-called microspecies, which differ comparatively little from each other but which do, nevertheless, clearly and constantly diverge. The populations of these apomictic microspecies are morphologically very homogeneous and variation among them is very slight. The reasons for this are the same as were shown above to set a limit to the variation and evolution among parthenogenetic weevils. A closer examination of these generally very uniform apomictic plant "species", reveals, as a rule, the existence of morphologically different biotypes. It is not possible here to go into details of the complicated question of apomixis, but we must confine ourselves to a consideration of a few examples related to the subject of this investigation. For a more extensive review of apomixis, reference should be made to the detailed summary by GUSTAFSSON (1946, 1947 a and b) with its reference list.

It has been found that in many cases the polymorphism exhibited by apomictic plants depends on differences in chromosome number. Divergent apomictic biotypes may thus represent different degrees of polyploidy or they may be aneuploid chromosome aberrants. Of numerous cases of this type, the apomictic Taraxaca may serve as an example here. SØRENSEN and GUDJÓNSSON (1946) have studied the spontaneous aberrants in apomictic Taraxaca of the *Vulgaria* group (2n=24). The aberrants, which are analogous for a number of microspecies, comprise two categories, 1) aneuploid chromosome aberrants (2n=23), of which eight morphologically well separated types are found — eight is the basic chromosome number in *Taraxacum* — and 2) polyploid aberrants. The primary aberrants may produce secondary aberrants, etc. SØRENSEN and GUDJÓNSSON assume that the chromosomal aberration in *Taraxacum* is probably due in part to abnormal mitoses, but it may possibly also be brought about during the formation of the embryo sac, even though typical meiosis is lacking. GUSTAFSSON (1935, 1947 b) has likewise studied the corresponding phenomenon in the apomictic Taraxaca and found it to be due to autosegregation. According to him (1947 b, pp. 188 ff.), in the simplest cases of autosegregation, "the division of the EMC proceeds irregularly, so that the daughter cells receive one chromosome too few or one too many. In *Taraxacum*, for instance, the female restitution nucleus may not incorporate all the twenty-four univalents scattered over the spindle. A similar exclusion of one or two chromosomes is easily imagined also for the pseudohomeotypic metaphase." In addition, the fact that biva-

lents are occasionally formed both in the formation of restitution nuclei and in the pseudohomeotypic metaphase, is sufficient to effect a segregation independent of fertilization, which also leads to the appearance of new, closely related biotypes. In the totally apomictic *Taraxacum* strains cultivated by KAPPERT (1954), aberrant specimens occasionally occurred, even in great numbers. Cytological examination revealed that these generally had an aberrant chromosome number. KAPPERT also supposes (p. 334), that in totally apomictic Taraxaca gene recombination, too, has been made possible to some extent by autosegregation. "Damit erhalten sich diese Totalapomikten eine gewisse Plastizität des Erbtypus."

In his investigations on the variation in apomictic microspecies of *Alchemilla vulgaris*, TURESSON (1956) has found that these microspecies are genetically very heterogeneous and composed of a number of apomictic biotypes. He also comes to the conclusion that the differentiation of the apomictic biotypes in *Alchemilla* is probably due to autosegregation.

When studying the polymorphism in apomictic *Poa alpina*, MÜNTZING (1954) established that "in all . . . cases the morphologically deviating plants were also cytologically deviating, the aberrant morphology being accompanied and certainly caused by the changed chromosomal constitution" (p. 465). When differences between progenies were slight, there was generally identity between chromosome numbers (p. 469). MÜNTZING concludes that "the most obvious source of new biotypes . . . in . . . more or less apomictic species of *Poa* is probably the occasional occurrence of fertilization of reduced or unreduced egg cells leading to the appearance of morphologically and cytologically deviating aberrants" (p. 506), and further, that "the polymorphism has slowly and gradually been increased by mutations affecting a small fraction of the enormous seed material formed each year" (p. 507).

In his investigations on the polymorphism in apomictic species of *Potentilla*, MÜNTZING (1958) has shown that although different biotypes having apomictic seed formation are highly constant, new biotypes can be formed in them not only by spontaneous chromosome doubling but also by reversions. These new biotypes are in part different *inter se* and they may also differ from the original mother strain. MÜNTZING assumes that new biotypes of *Potentilla collina* are continuously being formed in nature by spontaneous chromosome doubling and reversions and that "this process is a powerful means of increasing the polymorphism of the species" (pp. 318—319). That polymorphism can be caused by

reversions is attributed by MÜNTZING to the fact that "bivalents in the 84-chromosome type ('tetraploid') are not always formed by the same chromosomes". This, in turn, is due to the fact that "there is a strong degree of differential affinity or a random formation of bivalents between more or less homologous chromosomes" (p. 319).

Without recourse to further examples and without going into greater detail, we can, according to GUSTAFSSON (1947 b, p. 184) draw the conclusion that "the different methods by which new biotypes result in apomictic genera include the following processes: 1) hybridization and segregation, when fertilization is still able to occur, 2) polyploidization ... and its reverse procedure 'haploidization', 3) mutation and, finally, 4) autosegregation, the last two processes also taking place in such closed systems as those in which fertilization is totally absent".

4. Evolutionary possibilities among the polyploid parthenogenetic weevils

Of the above-mentioned four different methods which render possible the formation of new biotypes in apomictic plants, 1) and 4) are out of the question in such forms as the polyploid parthenogenetic weevils. Hybridization and segregation cannot take place in them, (see however below) since their parthenogenesis is obligatory and apomictic, and their ova thus undergo only one equational maturation division. Similarly, autosegregation cannot occur in them either, because the parthenogenetic animals in question do not possess the types of maturation division found in apomictic plants, (the formation of restitution nuclei and the pseudohomeotypic metaphase, cf. GUSTAFSSON, 1935, pp. 80—81) which make autosegregation possible. Although polyploidy is an important evolutionary factor among the parthenogenetic weevils, it is the differentiation of populations with the same degree of polyploidy with which we are primarily concerned here.

The weevil populations with the same degree of polyploidy do not exhibit any differences in chromosome number, which thus cannot be the cause of the observed differentiation of populations. Although there are occasional specimens with a deviating chromosome number in some polyploid parthenogenetic weevil populations (see, e.g., SUOMALAINEN, 1940 b, pp. 70—72, 78—79, 89—90), no such differences were found between the populations studied. The causes of differentiation, therefore, have to be sought elsewhere.

Of the different methods mentioned above there thus remain only mutations, including both gene mutations and structural chromosomal

changes. HUSKINS (1941) has suggested that in most polyploid species chromosomal changes are a more important source of variation than gene mutations. However, as we have mentioned already on pages 331—332, it is very difficult for a mutation to gain expression in forms like the polyploid parthenogenetic weevils. How, then, can the differentiation of their populations be based on mutations? With our present knowledge, only some speculations can be made here.

Various investigators (see, for instance, WHITE, 1945, p. 283; 1954, p. 341; GUSTAFSSON, 1947 b, p. 187; SUOMALAINEN, 1950, p. 220; SMITH, 1955, p. 132) have pointed out that the degree of heterozygosity steadily increases in apomictically parthenogenetic animals. If a gene mutation or structural rearrangement takes place in such an animal, the heterozygosity thus established is maintained in the following generations, because the mutation in these animals cannot become homozygous. Such animals become gradually heterozygous in regard to more and more gene pairs, since the elimination of recessive mutations by natural selection is impossible. So in animals which have been apomictically parthenogenetic for a long time, differences in the two chromosome sets have accumulated to such an extent that, in fact, they can no longer be considered diploid in either a cytological or a genetical sense (WHITE, l. cit.). The same also holds true with regard to polyploid forms of corresponding kind. They too will become, in a similar manner, more and more heterozygous, which in turn will reduce their polyploid character. It seems obvious that this will also reduce the obstacles to the expression of the mutations present in them and may thus in part even allow the formation of morphologically divergent biotypes, particularly as it has been assumed (GUSTAFSSON, l. cit.) that high heterozygosity in apomictic complexes "will help to augment the spontaneous mutability".

In addition, it is to be noted that if the degree of heterozygosity continuously increases, an apomictically parthenogenetic form gets an ever-increasing chance to benefit from heterosis. It is obvious that it is this phenomenon which, together with polyploid superiority, is one important cause of the generally wide distribution of polyploid parthenogenetic forms.

As mentioned on page 332 the inhibitory effect of polyploidy on the expression of dominant mutations is not as great as on that of recessive mutations. Therefore it seems obvious that dominant mutations have a rôle to play in the differentiation of polyploid parthenogenetic weevil populations.

Somatic crossing over in the oogonial phase and during the equa-

tional maturation division also offers a chance of differentiation in the polyploid parthenogenetic weevils. There are no observations on somatic crossing over in the weevils, however. In addition, and in contrast to those forms showing somatic crossing over (cf. SWANSON, 1957, pp. 268—269), no unusual synapsis between homologous chromosomes in the somatic cells has been found in the weevils.

Although the parthenogenesis of the weevils is obligatory, it may be suggested, on theoretical grounds, at least, that hybridization plays a rôle in the polymorphism of the polyploid parthenogenetic weevils. It is obvious that in these animals parthenogenesis evolved first and polyploidy followed. The origin of polyploidy in the parthenogenetic weevils, in turn, is most easily explained as the result of occasional hybridization between bisexual and parthenogenetic forms (SUOMALAINEN, 1947, pp. 448—450). Fertilization of an egg of a diploid parthenogenetic weevil by haploid sperm will give rise to a triploid race and fertilization of a triploid egg will produce a tetraploid. In their studies on apomictic *Potentilla* species, A. and G. MÜNTZING (1941) showed how greatly an occasional hybridization may increase polymorphism. They have seen that "occasional hybrids between different types within the same collective species may entirely loose their apomictic mode of propagation and produce swarms of new biotypes which may become stabilized in later generations" (p. 274). If the above-mentioned hypothetical hybridization of a bisexual and a parthenogenetic weevil race had taken place several times between the same races, the formation of different biotypes with the same degree of polyploidy would naturally have been the result. Nowadays, however, the chances of a repeated hybridization of a bisexual and a parthenogenetic race of the same species are very slight, since in most species the bisexual race — if it exists at all — has a very limited distribution. If such a hybridization could take place between different species, the chances of its occurring would naturally be increased. In any case, hybridization is so rare an event among weevils that the rôle it plays in polymorphism must be slight.

In forms like the polyploid parthenogenetic weevils, natural selection is incapable of eliminating deleterious recessive mutants since these cannot, as a rule, become homozygous. Yet it need not lose its significance in the evolution of organisms like these. Its eliminating effect will not be directed at individuals, in the usual way, but at populations, provided that such populations are able to differentiate genotypically.

WHITE (1954, p. 343) has called attention to the fact that a large proportion of the known cases of thelytoky in animals are probably so

recent in origin that there has been insufficient time for their evolutionary differentiation into agamic complexes. In his study on the distribution of the different races of *Solenobia triquetrella*, SEILER (1946) has come to the conclusion that in this bagworm moth both parthenogenesis and polyploidy arose — in this order — in the Alps during the last glacial period, the Würm Ice Age. The parthenogenesis owes its origin to the period at which the Würm glacier was at its widest, and the tetraploidy arose during the retreat of the glacier. Since in the Alps, at least, the distribution of the different races of some weevils resembles in principle the distribution of the respective races of *Solenobia*, we might from this draw the conclusion that in some, at least, of the weevils polyploidy and parthenogenesis have also arisen in the same order during the last Ice Age. The time elapsed since then is not very long, as the thawing of the glacier in Northern Europe, at least, began only about 12,000 years ago. SYLVESTER-BRADLEY (1959) has stressed the view that "if an apomictic population is to be considered in an evolutionary setting, it must be within a framework the time unit of which is not less than 50,000 years" (p. 173). This may be a partial explanation of the fact that the differences between populations of parthenogenetic weevils are no greater than at present.

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SUMMARY

In this investigation, the total length of the animal, the length of the head and thorax combined, the maximum breadth, maximum breadth of the thorax, maximum height, and length of both hind tibiae have been measured from specimens drawn from several different populations of each of five parthenogenetic weevil species (*Otiorrhynchus scaber*, *O. chrysocomus*, *O. salicis*, *O. singularis*, and *Peritelus hirticornis*), the eyepiece micrometer of a stereomicroscope being used. All populations studied were polyploid (triploid or tetraploid). The populations of the weevil species studied were compared with each other by calculating the ratio between the total length and each of the other

measurements successively. An analysis of variance, which included all populations, was performed with regard to each ratio.

Tetraploid weevil specimens appeared to be distinctly bigger than triploid specimens of the same species, although the difference was not great.

The above analysis has shown that different populations of the same parthenogenetic weevil species having the same degree of polyploidy exhibit significant and in some cases great morphological differences. Thus, for example, most of the Central European populations of *Otiorrhynchus scaber* investigated differ clearly from most of the Fennoscandian populations. Furthermore, among the latter, the populations of Porvoo and Kuujärvi are decidedly different from the other populations in Southern Finland.

It seems very probable that the differences between weevil populations detected in this investigation owe their presence, at least to a considerable degree, to differences in the gene pools of the populations. This indicates that, contrary to the earlier assumptions, evolution does not come to a complete standstill in polyploid parthenogenetic populations.

The possibilities of evolution in polyploid parthenogenetic weevils are discussed.

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SELECTION FOR SIZE IN MICE IN DIFFERENT NUTRITIONAL ENVIRONMENTS

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I. INTRODUCTION. MATERIAL AND METHODS

THE influence of the environment on the results obtained by selection constitutes an important problem for animal breeders. Not only the effect on the magnitude of the improvement but also the changes in correlated characters by selection for one character may be of importance.

The object of the selection experiment described in this paper was to study the results of selection for body weight on different planes of nutrition. Mice (*Mus musculus* L.) were chosen for the experimental material. FALCONER and LATYSZEWSKI (1952) have earlier published results of a similar investigation, but the differences between the investigations are considerable. It should be particularly emphasized that the difference between the planes of nutrition is much greater in this experiment than in that of FALCONER and LATYSZEWSKI. It should also be remembered that selection experiments in small populations can give different results that are entirely fortuitous. Therefore, a great number of experiments are required to illustrate this problem.

The foundation stock consisted of mice from two strains which the author had used in another selection experiment (KORKMAN, 1957). Fifteen animals from one of these strains were mated to fifteen animals from the other strain. From each of the fifteen litters born (generation P), one male and one female were kept on ad lib. feeding (generation HO), and one male and one female on a restricted diet (generation LO). Thus, one strain on high plane of nutrition (H), and one strain on low plane of nutrition (L), were founded.

The animals on high plane of nutrition were fed on a particular mice-bread and wheat germs ad lib. The animals on low plane of nutrition were kept on a restricted diet according to the following scheme:

1. *During the mating period*, every alternate day, 3 g mice-bread; and the other days 2.5 g mice-bread and 1 g wheat germs.
2. *Pregnant females*, every day, 3 g mice-bread and 1 g wheat germs.
3. *The females during the suckling period* (20 days), mice-bread ad lib. but no wheat germs.
4. *The young animals*, at an age of 21—24 days, every day, 2 g mice-bread,
   The young animals, at an age of 25—40 days, every alternate day, 3 g mice-bread and 1 g wheat germs, and the other days no feed.
5. *Animals more than 40 days of age waiting to be mated*, every day, 3 g mice-bread.

The young were taken from their mothers 20 days after birth, and at the same time the males were isolated from the females. The animals were kept in glass cages, never more than six in each cage.

The mating system was identical in each strain. The selection was based on the weight of the animals at an age of 40 days. Fifteen pairs were mated in each generation, and the heaviest female and the heaviest male were taken from each litter to be parents of the next generation. The selection was in this way made only within litters, being based on the deviation from the litter mean of the animals of the same sex. Inbreeding was minimized by the choice of the least related individuals for mating.

The animals were paired for mating at an age of 40 days. The animals on the high plane of nutrition had at this age reached sexual maturity, and pregnancy occurred shortly after the males had been brought to the females. However, the animals on the low plane of nutrition showed in many cases delayed puberty, and in some cases the males and females were together for weeks without the females becoming pregnant. In a few cases, when mating had not led to pregnancy, the animals were mated more than once in order to keep the number of litters in the generations constant. All animals in all generations were weighed at an age of 40 days.

II. RESULTS

1. Survey of results

Fig. 1 gives a survey of the results of the selection. The figure shows the averages of the litter means of the weights of the females and males at the age of 40 days in the different strains and generations. The line

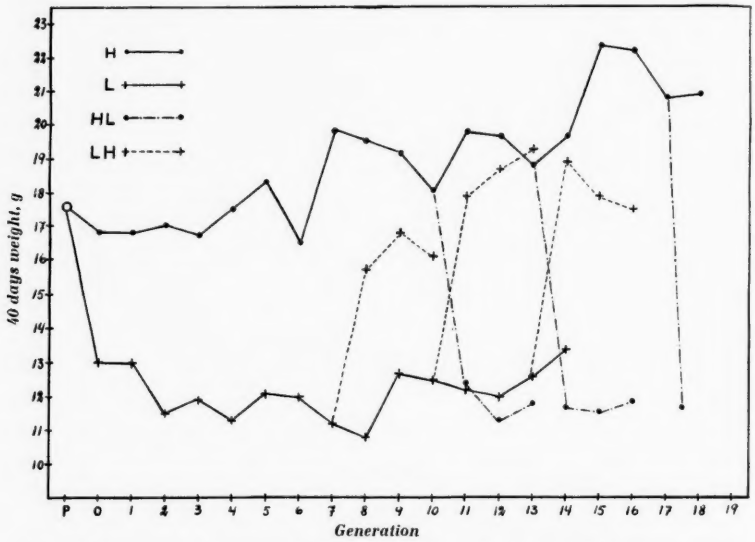

Fig. 1. Response to selection for 40 days weight in the strains H and L and to exchanged plane of nutrition.

marked H shows the generation averages of the population selected and raised on a high plane of nutrition, and the line marked L the corresponding averages of the population selected and raised on a low plane of nutrition. The lines marked HL show the generation averages of the populations selected on a high plane but raised on a low plane of nutrition; and the lines marked LH show the corresponding averages of the populations selected on a low plane but raised on a high plane of nutrition. Further information on the results is given in Tables 1, 2, 3 and 4.

The selection differential in each generation was obtained from the mean deviation of the parents from their litter-means of the animals of the same sex. The figures of the average weights in the litters and of the selection differentials are represented separately for each sex. The selection does not seem to have had any influence on the litter size at birth. The average size of the litters in strain H on the high plane of nutrition were 6.15, and in strain L on the low plane of nutrition, 6.12. The mortality of the young from birth to 40 days of age is, however, greater in strain L than in strain H, this difference being especially marked as regards the males. The average number of animals dying during the mentioned period was per litter:

TABLE 1. *The H strain. Selection differential, litter size and weight of the young at 40 days of age.*

Generation	Selection differential g		Litter size at birth	Weight at 40 days of age g	
	females	males		females	males
P	—	—	5.69	16.34	18.70
0	—	—	6.60	15.34	18.18
H 1	1.58	1.76	6.73	15.63	17.97
H 2	1.43	1.74	6.26	15.85	18.14
H 3	1.32	1.19	6.67	15.05	18.33
H 4	1.51	1.55	6.40	16.15	18.81
H 5	1.43	2.21	5.74	17.16	19.67
H 6	1.21	1.03	6.80	16.21	16.78
H 7	1.44	1.11	5.54	17.85	21.81
H 8	1.11	1.78	6.40	18.28	20.85
H 9	1.75	1.23	6.60	17.70	20.67
H 10	0.79	0.86	5.67	16.66	19.43
H 11	1.13	1.51	6.13	18.68	21.01
H 12	1.03	2.20	6.47	18.75	20.72
H 13	1.12	1.23	6.10	17.73	20.14
H 14	1.00	1.13	5.73	18.57	20.77
H 15	0.99	1.22	5.40	21.09	23.70
H 16	0.77	0.66	6.00	20.96	23.44
H 17	0.51	1.77	6.00	19.71	21.96
H 18	1.03	1.31	6.00	19.76	21.97
Average	1.18	1.42	6.15	17.88	20.34

in strain H 0.10 females and 0.09 males and
„ „ L 0.17 „ „ 0.22 „ .

This difference in mortality between strains H and L is in regard to the males, strongly significant ($\chi^2=14.64$; $P<0.001$). Also the difference between the mortality rates for females is significant ($\chi^2=5.08$; $P<0.05$). However, the males seem to be more affected by the environment than the females.

2. Response to selection

The response to the selection in the strain H was calculated as follows. The mean of the selection differentials of females and males in the generation was used in the calculations, and these mean differentials of successive generations were added together to obtain the cumulative

TABLE 2. *The L strain. Selection differential, litter size and weight of the young at 40 days of age.*

Generation	Selection differential g		Litter size at birth	Weight at 40 days of age g	
	females	males		females	males
P	—	—	5.69	16.34	18.70
0	—	—	6.26	12.17	13.75
L 1	1.05	1.31	5.79	12.44	13.53
L 2	1.22	1.59	6.45	11.14	11.80
L 3	0.81	1.33	6.50	11.57	12.28
L 4	0.48	1.70	6.11	11.07	11.49
L 5	1.35	1.49	5.50	11.93	12.25
L 6	0.99	1.12	6.39	11.71	12.21
L 7	0.47	1.53	6.00	11.12	11.31
L 8	0.93	0.47	6.60	10.82	10.85
L 9	1.14	1.10	6.46	12.45	13.04
L 10	0.60	0.57	6.00	12.27	12.72
L 11	0.95	1.17	6.13	11.81	12.65
L 12	0.89	1.23	6.46	11.80	12.20
L 13	0.35	0.20	5.67	12.06	13.09
L 14	0.52	1.92	6.60	13.06	13.82
Average	0.78	1.20	6.12	11.80	12.37

differential, which showed the progress that would have been made if the heritability had been 100 per cent. These cumulative values of the selection differentials (z) were correlated with the order number of the generation (x).

The regression of the cumulative selection differential on the generation number (b_{zx}) was found to be 1.326 and the corresponding coefficient of correlation was 0.999 ($P<0.001$).

The response to selection was measured by the average of the litter-means of the weights of the females and of the males. The average (y) obtained for each generation was also correlated with the order number of the generation (x). The regression of the response on the generation number (b_{yx}) was found to be 0.289 and the corresponding coefficient of correlation was 0.853 ($P<0.001$).

The heritability was calculated as the ratio of the regression coefficient of the response to that of the selection. Thus, $h^2=\frac{0.289}{1\,326}=0.218$. The coefficient of correlation of 0.999 shows that the variance in b_{zx} is

TABLE 3. *The LH strain. Selection differential, litter size and weight of the young at 40 days of age.*

Generation	Selection differential g		Litter size at birth	Weight at 40 days of age g	
	females	males		females	males
L 7 H 1	0.49	0.15	6.27	14.53	16.61
L 7 H 2	1.07	1.53	5.93	16.11	17.48
L 7 H 3	1.53	1.53	6.87	15.15	17.03
L 10 H 1	0.61	0.83	6.00	17.37	18.35
L 10 H 2	0.48	1.73	6.47	17.15	20.18
L 10 H 3	— 0.16	— 0.37	6.20	17.61	20.92
L 13 H 1	0.45	0.57	5.80	17.61	20.27
L 13 H 2	0.15	0.71	5.47	16.75	19.04
L 13 H 3	0.17	— 0.68	5.93	16.48	18.51
Average	0.53	0.67	6.10	16.53	18.71

very small, and therefore the probability that the heritability differs from zero will be about the same as the probability that b_{yx} differs from zero.

The results from strain L on the low plane of nutrition gives the corresponding values $b_{yx}=0.065$; $r_{xy}=0.371$ ($P<0.2$) and $b_{zx}=0.996$; $r_{xz}=$ $=0.996$ ($P<0.001$). The heritability will be calculated as $\frac{0.065}{0.996}=0.066$ ($P<0.2$). Thus, no significant improvement of the weight has been

TABLE 4. *The HL strain. Selection differential, litter size and weight of the young at 40 days of age.*

Generation	Selection differential g		Litter size at birth	Weight at 40 days at age g	
	females	males		females	males
H 10 L 1	0.91	1.26	5.47	12.21	12.41
H 10 L 2	0.79	1.06	4.73	11.72	10.95
H 10 L 3	0.50	0.83	6.53	11.72	11.92
H 13 L 1	1.13	0.98	6.13	11.21	12.20
H 13 L 2	0.28	— 0.35	6.13	11.41	11.81
H 13 L 3	0.04	— 0.01	5.53	11.92	11.88
H 16 L 1	0.21	0.87	5.80	11.60	11.93
Average	0.55	0.66	5.76	11.68	11.87

observed. However, Fig. 1 indicates that the weight of the animals has decreased in the 1st to the 8th generations and then perhaps to some extent increased.

3. Results of changes in environment

Changes in environment were made in strain L in the 7th, 10th and 13th generations. In every case fifteen females and fifteen males were changed to a high plane of nutrition (strains LH), and three generations of these populations were then raised on a high plane of nutrition. Fig. 1 and Table 3 show the results. In the 10th, 13th and 16th generations of strain H the corresponding number of animals were changed to a low plane of nutrition (strains HL). The populations, changed from the 10th and 13th generations, were also raised in three generations on a low plane of nutrition, but the population, changed from the 16th generation, was only raised on a low plane of nutrition in one generation because the whole experiment was then concluded. Fig. 1 and Table 4 show the results.

The weight of the animals at an age of 40 days was on an average lower in the LH populations than in strain H on a high plane of nutrition. And the individuals in the HL populations showed a lower weight at an age of 40 days than the animals in strain L on a low plane of nutrition.

The significance of these differences has been tested by comparing the weight of the animals in the LH strains with that in the 7th to 14th generations of strain H, and by comparing the weight of the animals in the HL strains with that in the 10th to 14th generations of strain L. In both cases an equal number of generations has been compared, but the order number of the generations in the H and L strains are one unity lower than in the corresponding LH and HL strains. This has been done because the selection has been in some degree smaller in the LH and HL strains than in the corresponding generations of H and L, the comparability being therefore better in this way. Table 5 shows the results of these comparisons.

Some comments on this test of significance may be relevant here. The compared groups are not quite contemporaneous, and therefore there can exist some other environmental differences between the groups. However, the probability that these differences should have a marked influence seems to be small.

TABLE 5. *The differences between the H strain and LH strain and between the L strain and HL strain.*

Strain and sex	Number of animals	Mean weight g	s g	Difference between the means	P
H females	360	18.036	2.230	1.540	<0.001
LH „	340	16.496	2.780		
H males	344	20.558	3.159	2.041	<0.001
LH „	352	18.517	3.557		
L females	211	12.283	1.815	0.784	<0.001
HL „	191	11.499	1.483		
L males	211	12.968	1.716	0.956	<0.001
HL „	182	12.012	1.397		

4. Correlation between parents and offspring

The correlation between the weight of the parents (x) at an age of 40 days and that of the offspring (y) has been studied separately in the H and L strains, and in both cases the coefficients of correlation and regression have been estimated within generations and sexes. In the H strain the correlation is 0.066, and the regression of the offspring on the parents 0.076. Thus an estimation of the heritability on the basis of the coefficient of regression gives $h^2=0.152$. This value of h^2 is lower than the earlier value of 0.218, estimated on the basis of the response to selection. The difference is obviously caused by differences between the methods. In the earlier estimate the deviation of the parents from the litter-mean has been used, and in the latter the weight of the parents. The weight of animals is apparently more influenced by environmental differences between litters than the deviation from the litter-mean. The breeders have to pay attention to similar conditions, *e.g.* by selection on the basis of milk yield in cattle. The deviation from the corresponding herd average is a better estimate of the breeding value than the actual yield.

In the L strain the corresponding coefficients of correlation and regression are 0.029 and 0.034, and the estimated value of heritability is 0.068. This value of h^2 corresponds fairly well with the earlier value of 0.066, calculated on the basis of the response to selection. However, neither of the two values is significant.

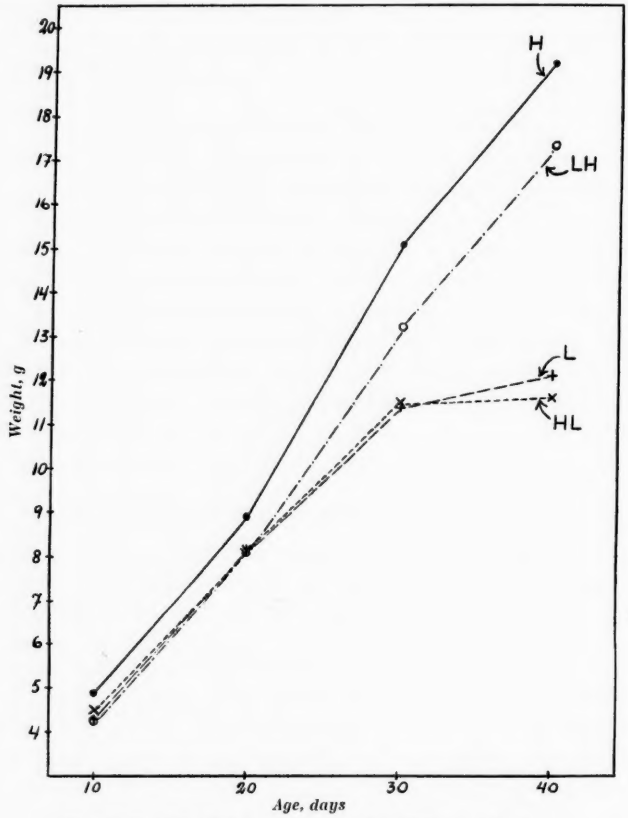

Fig. 2. Growth of the animals in the different strains.

5. Growth

In the first generations the animals were weighed only once at 40 days of age, but beginning with the generations H 5 and L 4 all animals were weighed at 10, 20, 30 and 40 days of age. Fig. 2 shows the growth of the animals during this period in the different strains. The curve H represents the averages of the litter-means of females and males of the generations 5—18, and the curve L the corresponding averages of the generations 4—14. In the HL and LH curves those generations in which the parents have been raised on another plane of nutrition have not been included. Therefore, the curve HL represents

the averages of the generations H 10 L 2, H 10 L 3, H 13 L 2 and H 13 L 3, and the curve LH the averages of the generations L 7 H 2, L 7 H 3, L 10 H 2, L 10 H 3, L 13 H 2 and L 13 H 3.

The weight of the animals at 40 days of age has been discussed earlier in this paper, but the curves in Fig. 2 give more information on the differences between the strains. Fig. 2 shows that the individuals in the H strain have been heavier on an average than the individuals in the other strains at the time of all weighings. Thus, the animals in the H strain have been heavier than those in the LH strains although the latter, too, have been raised on a high plane of nutrition. Table 5 shows that the difference between H and LH is significant at 40 days of age.

At 10 and 20 days the animals in the L and LH strains have been somewhat lighter than the animals in HL. However, the differences are not significant, and already at 30 days the animals in LH are considerably heavier. At 10, 20 and 30 days the animals in L and HL are almost equal in weight, but at 40 days the animals in HL are significantly lighter than the animals in L (Table 5). This observation indicates that animals from a population selected on a high plane of nutrition are not as well adapted to a very low plane of nutrition as animals from a population selected on this low plane.

6. Correlations between body length, body weight and tail length

In a number of generations the body length (from the tip of the nose to the base of the tail) and the tail length were measured at 40 days of age. Information on these characters is available from the H 13—H 16, L 10—L 14, H 13 L 1, H 13 L 2, H 13 L 3, H 16 L 1, L 10 H 1, L 10 H 2, L 10 H 3, L 13 H 1, and L 13 H 2 generations. Each of strains H, L, HL and LH has been treated as a whole and has not been divided into sex groups nor into generation groups. The curves in Fig. 3 show the frequencies of animals in the different body length classes.

Fig. 3 shows great differences between H and LH and also between L and LH. On the other hand, in L and HL the number of animals is most equal in the different body length classes. Thus, the body length at 40 days of age is on an average the same in L and HL. However, it has earlier been demonstrated that the body weight at 40 days of age is significantly smaller in HL than in L.

On account of the surprising fact just mentioned it will be appropriate to present some additional observations on the body weight and body

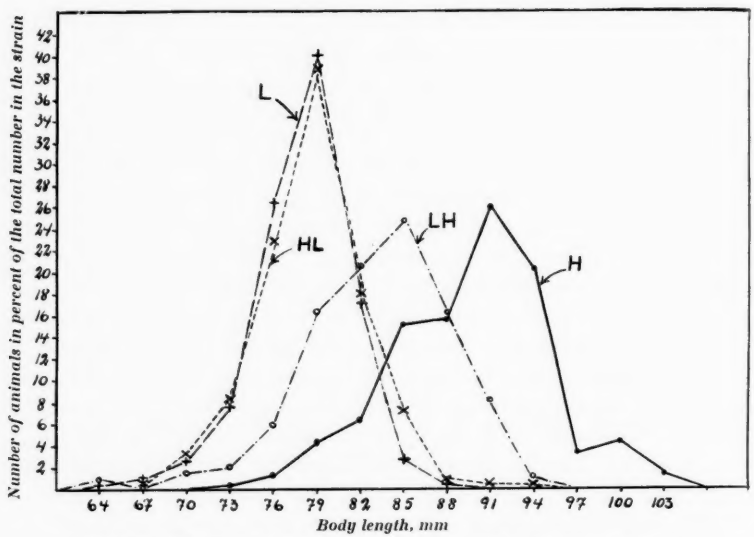

Fig. 3. Distribution of the animals on different body length classes.

length, and on the correlation between these two characters in the different strains (Table 6).

Table 6 also gives information on the tail length and on the correlation between body length and tail length in the different strains. Fig. 4 shows the regressions of the body weight and the tail length on the body length. Only class averages representing at least five individuals are included in the figures. The regression lines seem to be very nearly rectilinear and on this assumption analyses of covariance have been made in order to study the differences between the regression lines. The

TABLE 6. *Body weight, body length and tail length in the different strains.*

Strain	Body length (x) mm	Tail length (y) mm	Body weight (z) g	Regression b_{yx}	Regression b_{zx}
H	89.9	70.1	20.7	0.57	0.56
L	78.2	62.0	12.6	0.36	0.30
HL	78.6	63.5	11.7	0.46	0.26
LH	83.5	63.3	18.5	0.55	0.56

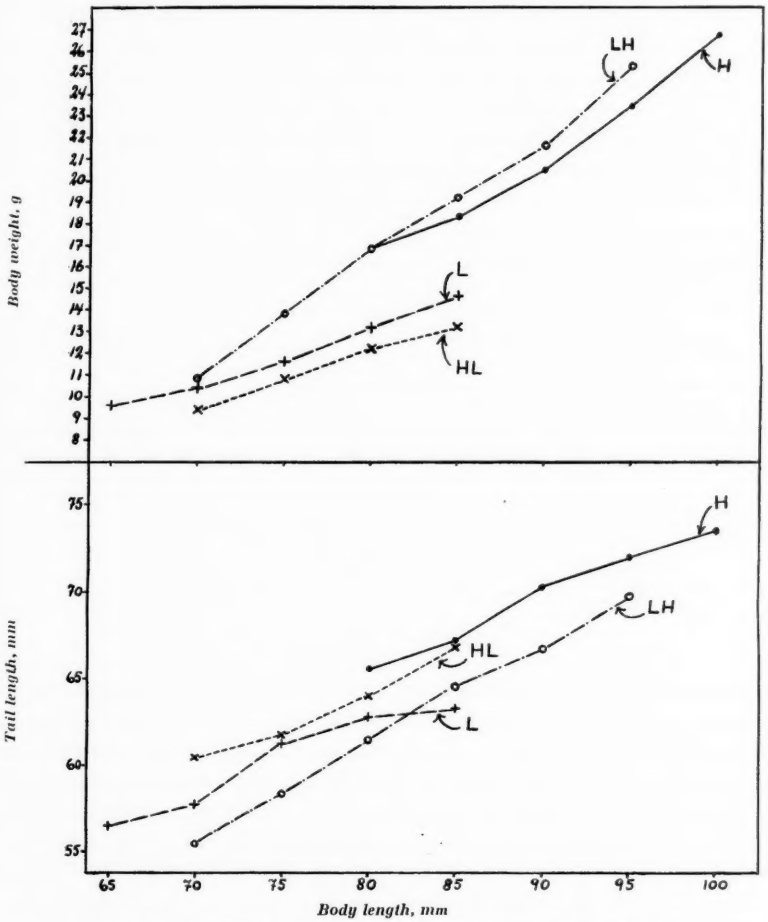

Fig. 4. Regression of body weight and tail length on body length.

analyses have been made according to the method presented in Table 7. In these calculations 1 mm and 0.5 g have been used as units. Table 8 shows the results of these investigations.

In regard to the regression lines of the body weight on the body length the differences between all strains are significant. It is of interest to establish the fact that the individuals in HL originating from a pop-

TABLE 7. *Differences between the H strain and the L strain with regard to regression of body weight on body length.*

Source of variance	D/f	Residual squares	Mean residual squares
Distribution of variates in relation to the total regression line (a)	741	9,938.15	
Distribution of variates in relation to the parallel regression lines within strains (b)	740	7,852.29	10.61
Error a—b=difference between the two strains in regard to their lines of regression	1	2,085.86	2,085.86
Distribution of variates in relation to the regression lines of each strain (c)	739	6,988.09	9.46
Errer b—c=difference between the two strains in regard to the regression coefficients	1	864.20	864.20

Quotient $\frac{2{,}085.86}{10.61}=196.59$ ($P<0.001$)

Quotient $\frac{864.20}{9.46}=91.29$ ($P<0.001$)

ulation selected on a high plane of nutrition but raised on a low plane, are, in proportion to their body weight, longer than the individuals in L, originating from a population selected on a low plane of nutrition and raised on the same plane. On the other hand, the individuals in LH are in proportion to their body weight shorter than the individuals in H. In this case the animals have been raised on a high plane of nutrition but strain LH has been selected on a low plane. Selection on a high plane of nutrition seems to favour longer animals, and probably the efficiency of feed utilization will be more improved by selection on a very low plane of nutrition.

The differences between the coefficients of regression have significant values only in comparing populations on different planes of nutrition. The difference between the coefficients of H and LH is not significant, nor is the difference between L and HL. The results also indicate that selection on different planes of nutrition has an influence on the regression of the tail length on the body length. Selection on a high plane of nutrition seems to favour animals with longer tails.

TABLE 8. *Significance of the differences between the strains with regard to the regression of body weight on body length and the regression of tail length on body length tested according to the method described in Table 7.*

Correlated characters and compaired strains	Quotient $\frac{\text{mean residual spuare (a−b)}}{\text{mean residual square (b)}}$	Quotient $\frac{\text{mean residual square (b−c)}}{\text{mean residual square (c)}}$
Body length—body weight:		
H+L	196.59 ($P<0.001$)	91.29 ($P<0.001$)
H+HL	301.93 ($P<0.001$)	87.62 ($P<0.001$)
H+LH	62.92 ($P<0.001$)	N. S.
L+HL	132.71 ($P<0.001$)	N. S.
L+LH	660.44 ($P<0.001$)	101.53 ($P<0.001$)
HL+LH	908.25 ($P<0.001$)	97.69 ($P<0.001$)
Body length—tail length:		
H+L	25.90 ($P<0.001$)	10.30 ($P<0.01$)
H+HL	N. S.	N. S.
H+LH	76.18 ($P<0.001$)	N. S.
L+HL	19.87 ($P<0.001$)	N. S.
L+LH	13.86 ($P<0.001$)	8.11 ($P<0.01$)
HL+LH	52.78 ($P<0.001$)	N. S.

III. DISCUSSION

The difference between the two planes of nutrition in the selection experiment described in this paper is much greater than in the earlier experiment of FALCONER and LATYSZEWSKI (1952). The lower plane is extraordinarily low. The weight of the young seems in many cases to decrease during the period between 30 and 40 days of age. This difference between the experiments probably causes the difference between the results. Nevertheless, there also exist other differences between the experiments.

In fact, FALCONER and LATYSZEWSKI found that heritability of the body weight was higher in selection on a low plane of nutrition (0.291) than in selection on a high plane (0.197). The reverse is the case in the experiment now described, which shows that the heritability is lower (0.066) in selection on a low plane and higher (0.218) in selection on a high plane. In both experiments, however, the performance was best improved by selection on that plane of nutrition on which the performance was subsequently measured.

LAGERWALL (1958) made an experiment on the effects of the plane

of nutrition on different characters, and the results seemed to indicate that an adaptation to small litters had occurred in the line on the low nutrition plane. The results of the experiment now described do not verify the findings of LAGERWALL. The litter size is on an average the same in strains H and L.

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SUMMARY

A selection experiment with mice has been made in order to show the influence of different planes of nutrition on the results of the selection. Two strains derived from a single population were selected for body weight at 40 days of age. The main results were:

(1) Weight increased in the population on a high plane of nutrition; the average increase corresponded to a heritability of 0.22.

(2) In regard to the weight no significant response to the selection was observed on the low plane of nutrition.

(3) Selection for body weight on a high plane of nutrition seemed to favour long and long-tailed animals.

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UNEQUAL CROSSING-OVER IN THE BAR REGION OF DROSOPHILA MELANOGASTER

II. INFLUENCE OF TEMPERATURE, X-RAYS AND EDTA

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I. INTRODUCTION

THE typical increase of crossing-over frequency found in the centromere regions of *Drosophila melanogaster* after treatment with high temperature, X-rays and γ-rays, is known to appear in broods from eggs laid 5—10 days after the treatment (MAVOR and SVENSON, 1924). Owing to this time lag, one has been forced to suppose that the treatment affects a pre-meiotic stage of the oogenesis. The increased crossing-over frequency has been attributed either to an indirect enhancing effect on the spontaneous meiotic crossing-over process (HERSKOWITZ and ABRAHAMSON, 1957), or to induced crossing-over events in gonial cells. These latter may subsequently divide mitotically one or more times before the meiosis and produce clusters of identical or reciprocal crossover types (WHITTINGHILL, 1955).

An investigation of the frequency of unequal crossing-over in the *Bar* region of the X-chromosome, which produces reverted wildtype and *double Bar* from homozygous *Bar* females, may give further information on this problem. For several reasons the phenomenon of unequal crossing-over is convenient for crossing-over studies. The *Bar* region is situated fairly near the centromere of the X-chromosome and can therefore be supposed to be influenced by the treatments, the effects of which seem to be limited to the vicinity of the centromere. The unequal crossing-over is a rare enough event (about one in 800 cases) to allow the detection of clusters, if such are produced, and may therefore allow a distinction between meiotic crossing-over and gonial crossing-over, leading to clusters of crossovers in the offspring and to inequality in number of reciprocal crossover types. By including marker genes on

each side of the *Bar* region the frequency of unequal crossing-over can be compared to the frequency of crossovers in the surrounding segments of the chromosome. A parallellism between these two frequencies would make it permissible to draw conclusions from one phenomenon to the other. Thus, if an increase of unequal crossovers could be shown to be due to clusters, and thus to gonial events, it could be assumed that a parallel increase in crossovers between *f* and *car* is also due to gonial crossing over.

In a pilot experiment (RASMUSON, 1957) it was found that treatment of *Drosophila* females with high temperature increased the frequency of unequal crossovers in the *Bar* region. This increase was parallelled by an increase of crossing-over in the adjacent segments *f—od* and *od—car*, and was limited to the later broods, oviposited 6 to 10 days after the treatment. However, in this limited material (including 74 cases of unequal crossovers) no clusters of equal or reciprocal crossover types were found which could have originated from a crossing-over event during the gonial stage.

The present investigation is an extension of these experiments to include X-ray treatment of females with two doses, 4000 r and 2500 r, and treatment with ethylenediamine tetra-acetic acid (EDTA or versene) during the larval stage. This compound is a chelating agent having the effect of depriving the cells of heavy cations, especially calcium. LEVINE (1955) and STEFFENSON (1956) have shown that this agent increases the crossing-over frequency in the entire length from *y* to *f* of the X-chromosome in females which during their larval stage were fed on substrate containing this compound. It was believed that a comparison between the effect of EDTA with that of high temperature and X-rays could throw further light on the causes of a frequency increase. The earlier experiments with high temperature treatment have been extended and are also included in the present report.

II. MATERIAL AND METHODS

The following mutant genes have been studied:

f	forked	X-56.7
B	Bar	X-57.0
BB	double Bar	X-57.0
od	outstretched	X-59.2
sy	small eyes	X-59.2
car	carnation	X-62.5

The stocks used are the same as in the pilot experiment, viz. *f B od sy car*; *B*; and *f od car*. The scored offspring has come from the cross between homozygous Bar females of the genotype *f B od sy car/B* and males *f od car*, where all the mutant genes (except *sy* in the daughters) are phenotypically expressed. Unequal pairing between the Bar segments in the *B/B* mothers in combination with crossing-over within the paired segment give rise to four different chromatid types, two with reverted B^+ (containing one segment 16A) and two with *double Bar* (containing 3 segments 16A), viz.: 1. *f* B^+; 2. B^+ *od sy car*; 3. *f BB*; 4. *BB od sy car*. The two types with round eyes can both be easily detected in the offspring, and are distinguished from the rarely occurring patroclinous males by combination with the mutant genes *f* or *od sy car*. Also, the *BB* females are readily distinguished from the heterozygous *B*/+ daughters and from exceptional non-disjunction *B/B* daughters. The non-disjunction females are characterized by their not expressing any recessive mutant characters. Among the male *BB* phenotypes, *f BB* is well distinguished from *f B*, but the occurrence of *sy* in the other type, *BB od sy car*, makes it sometimes difficult to distinguish from *B od sy car*, since also *sy* reduces the number of facets. Doubtful cases were outcrossed to wild-type females and tested by their female offspring, which should be all *BB*/+, if the father contained the *BB* gene. In those cases where offspring was obtained the preliminary classification was found to be correct.

Young virgin females of the genotype *f B od sy car/B* were collected, being either used untreated for the control crosses or being subjected to some treatment with the object of inducing a higher crossing-over frequency. The treatment with high temperature (31° C) was extended over 48 hours. The X-ray doses of 4000 r and 2500 r were delivered to the females when unetherized and fully motile. The radiation was performed in a Järnhs Skandia machine with an intensity of about 500 to 800 r per minute. A PTW Duplex dosimeter of type 1260/S_1 was used. Immediately after the treatment the females were distributed on vials, 5 females in each, and provided with males of the genotype *f od car*, two to five in each vial. The eggs laid by the females were separated in successive broods, including the oviposition of 2—3 days each. From two to five broods were raised in each experiment.

When EDTA was used, it was supplied to the substrate of larvae from the cross *f B od sy car/f B od sy car*×*B*. The larvae were fed on a suspension of yeast in 0.01-M EDTA and 0.04-M KOH, which STEFFENSON *et al.* (1956) have shown to be most effective in increasing the frequency

of crossing-over. At the time of hatching the virgin females were collected and used in the same way as described above for the other treatments.

Some special designations and symbols have been used in the following: All experiments where the animals have been subjected to the same treatment belong to one *series*, and within each series the experiments are numbered. The controls have been given the same number as the experiment with which they were contemporaneous, preceded by a K. T stands for the series of experiments where high temperature was used, R40 for the series exposed to 4000 r, R25 for the series exposed to 2500 r, and E for the series exposed to EDTA during the larval stage. UCO stands for unequal crossing-over or unequal crossovers in the Bar segment.

III. RESULTS

1. Frequency of crossovers in the region f — car in relation to age and treatment

The pilot experiment indicated an increase in crossing-over frequency, following high temperature treatment in the broods oviposited 6—10 days after the start of the exposure, for the region *f—od* as well as for *od—car*. Further, the well-known age effect of decreasing frequency was observed in the control. In the total material, therefore, the broods have been arranged according to age of mothers at oviposition and, in the temperature and X-ray treated series, also according to days after exposure. This period has in the T-series been counted from the middle of the 2-day treatment. Three intervals have been considered: 1) up to 5 days after treatment; 2) 5 to 10 days after treatment; 3) more than 10 days after treatment.

The results are shown in Table 1 and Figs. 1 and 2. Since no different response was indicated for the two regions *f—od* and *od—car*, only the results concerning the total region *f—car* are reported. The four control experiments give a joint picture of decreasing frequency up to the age of 8 days, followed by a stable period extending to the end of the experiments at 15 days. Heterogeneity among experiments is present in the earlier broods, but is not found during the stable stage. The crossing-over frequencies are mostly a little higher than the standard map distance, which is 5.8 %. Homozygosity for *Bar*, which implies a duplication for the seven bands of segment 16A, may account for this. In the figures the average control frequencies for different ages have been

TABLE 1. *Frequency of crossovers in the f—car region, and of UCO in the Bar segment for successive broods in controls and following different treatments.*

N=number of offspring scored for all mutants; N_1=number of offspring scored for UCO when this exceeds N.

Treatment	Exp. No.	Brood No.	Mean age of mothers	Days after treatment	N	Crossovers f — car		N_1	UCO	
						number	%		number	‰
Control	K2T	1	3		3902	310	7.94	4783	5	1.05
		2	6		4902	366	7.47	5890	11	1.87
		3	9.5		3387	214	6.32	3872	4	1.03
		total							20	1.38
	K3T	1	6		2569	172	6.70		2	0.78
		2	9		3798	212	5.58		9	2.37
		3	12		4568	299	6.55		2	0.44
		total							13	1.19
	K2R	1	4		646	58	8.98		0	0
		2	7.5		2583	147	5.69		6	2.32
		3	11		2532	172	6.79		2	0.79
		4	14		2348	146	6.22		5	2.13
		total							13	1.60
	K4R	1	2.5		1463	90	6.15		2	1.37
		2	6		1088	58	5.33		2	1.84
		3	8.5		1491	86	5.77		1	0.67
		4	11.5		1645	84	5.11		2	1.22
		5	15		1293	61	4.72		1	0.77
		total							8	1.15
31° C for 48 hours	T1	1	8	2—4	4296	295	6.87		4	0.93
		2	10.5	5—6	7327	498	6.80		14	1.91
		3	13	7—9	7032	473	6.73		10	1.42
		total							28	1.50
	T2	1	11	6—8	2795	203	7.26	3787	8	2.11
		2	14	9—11	3709	241	6.50	4770	12	2.52
		total							20	2.34
	T3	1	9.5	5—6	3004	238	7.92		9	3.00
		2	12	7—9	5218	379	7.26		11	2.11
		3	15	10—12	4868	275	5.65		6	1.23
		total							26	1.99
	T4	1	5	2—3	11732	841	7.17		19	1.62
		2	7	4—5	9985	663	6.64		8	0.80
		3	9.5	6—8	7427	468	6.30		12	1.62
		4	12	9—10	7906	555	7.02		17	2.15
		5	14	11—13	7392	409	5.53		19	2.57
		total							75	1.69

Treatment	Exp. No.	Brood No.	Mean age of mothers	Days after treatment	N	Crossovers f — car		N_1	UCO	
						number	%		number	‰
X-rays	R1	1	8	3—5	2571	179	6.96		3	1.17
4000 r		2	11	6—8	4191	259	6.18		7	1.67
		3	14	9—11	3808	211	5.54		8	2.10
		4	17.5	12—15	3826	287	7.50		13	3.40
		total							31	2.15
	R3	1	10.5	6—7	7249	455	6.28		15	2.07
		2	12.5	8—9	11937	647	5.42		14	1.17
		total							29	1.51
	R5	1	4	2—4	7501	492	6.56		11	1.47
		2	7	5—7	4771	341	7.15		14	2.93
		3	9.5	8—9	4597	293	6.37		8	1.74
		4	12	10—12	5532	379	6.85		12	2.17
		5	15	13—15	5089	324	6.37		9	1.77
		total							54	1.96
X-rays	R2	1	5	3—5	2643	177	6.70		4	1.51
2500 r		2	8	6—8	4279	292	6.82		3	0.70
		3	11	9—11	3980	237	5.95		5	1.26
		4	14	12—14	3226	191	5.92		7	2.17
		total							19	1.35
	R4	1	5.5	3—4	4867	321	6.60		8	1.64
		2	8	5—7	6556	414	6.31		6	0.92
		3	10.5	8—9	4193	213	5.08		6	1.43
		4	13	10—12	5624	347	6.17		3	0.53
		5	16	13—15	4347	254	5.84		3	0.69
		total							26	1.02
EDTA	E1	1	3		4859	297	6.11		4	0.82
+ KOH		2	5.5		5464	359	6.57		9	1.65
during		3	8		5105	304	5.95		6	1.18
larval		4	11		4446	239	5.38		9	2.02
stage		5	14		3178	233	7.33		7	2.20
		total							35	1.52
	E2	1	2.5		12057	720	5.93		12	1.00
		2	6		10256	593	5.78		9	0.88
		3	9		8175	484	5.92		7	0.86
		4	12.5		6048	416	6.88		11	1.82
		5	16		3766	246	6.53		5	1.33
		total							44	1.09

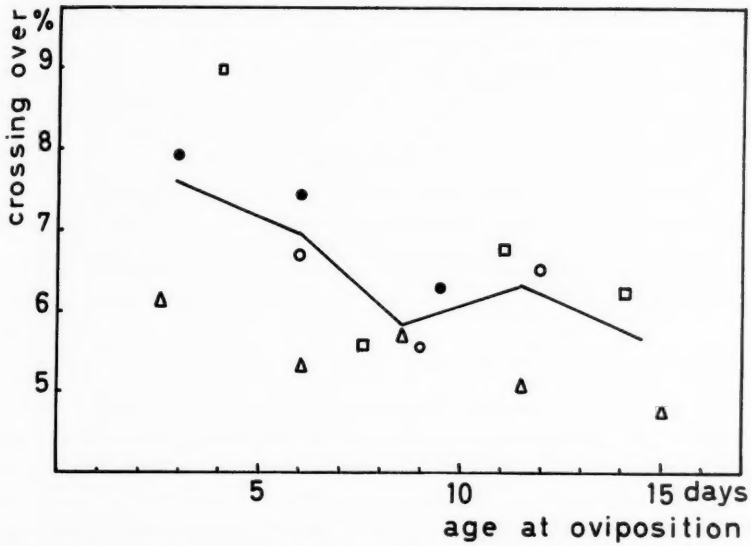

Fig. 1. Crossing-over frequency in the *f—car* region for the control series in relation to age at oviposition. Each sign is the frequency for one brood. Different signs are used for each of the four experiments. Average values are indicated by the line.

represented by a line (disregarding the fact that it is based on heterogeneous estimates in its first part). The values of separate broods are indicated by dots at the corresponding age of mothers. The three exposure-related intervals are indicated by different marks in Fig. 2.

The effect of the treatment is evident in the T-series, where a significant increase in crossover frequency can be seen during the middle period. No such increase is found either in the X-ray or in the EDTA treated series. The only discrepancy between the control and the R-series is an increase in the last interval of R40. The difference between this frequency, 6.9 %, and the control at the corresponding age, 5.7 %, is significant ($\chi^2_{het}=6.39$; $P<0.02$). There is also a significant difference between R40 and R25 in this last interval. In the E-series the trend differs from that of the control in being constant or slightly increasing throughout the observed broods. In the first broods the frequency is lower than that of the control, in the later broods significantly higher.

The observed effects of high temperature and high X-ray dose are in agreement with those found by MAVOR and SVENSON (1924) in that the effect of the temperature treatment is more strictly time-limited than

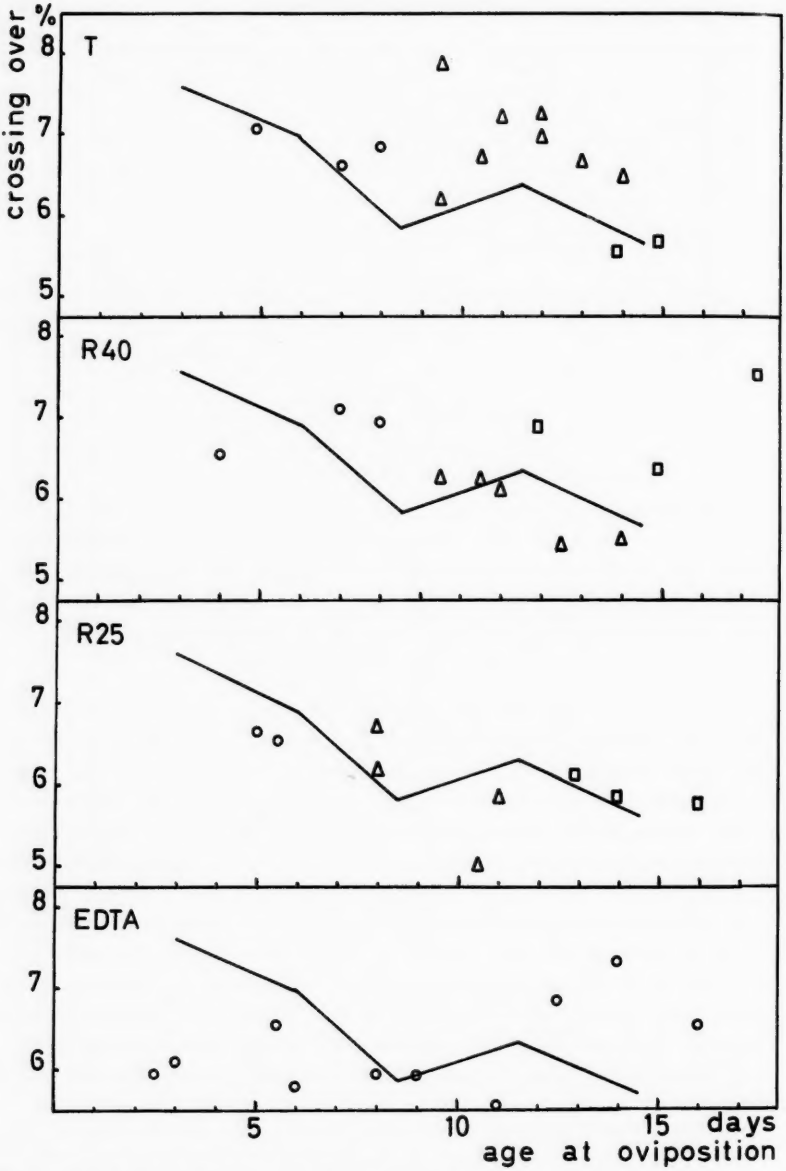

Fig. 2. Crossing-over frequency in the *f—car* region for the experimental series in relation to age at oviposition and time after treatment. Age at oviposition is given along the X axis. The time after treatment is indicated by different signs in the T and R series. ○ less than 5 days; △ 5—10 days; □ more than 10 days after treatment. For the EDTA series no subdivision is undertaken. The average control frequencies for different ages are indicated by the same line as in Fig. 1.

that of the X-ray treatment. The lower X-ray dose seems to be without effect in the region under observation. The effect of EDTA is rather dubious, and clearly less marked than that found in other experiments (LEVINE and STEFFENSON).

2. Frequency of unequal crossovers

The frequencies of unequal crossovers in different experiments and successive age-intervals following treatment have been analyzed for heterogeneity and significance of observed differences. Heterogeneity χ^2:s have been calculated using the formula $\chi^2 = \frac{[S(n)]^2}{S(a) \times S(n-a)} \left[S(a^2/n) - \frac{[S(a)]^2}{S(n)} \right]$, where a is the number of UCO in a group of n scored individuals. The degrees of freedom are one less than the number of groups. When a frequency difference between two groups is rated, $\sqrt{p}$; $p = a/n =$ the proportion of UCO; is taken as a normal deviate with variance 1/4n, and a t-value for the difference is calculated.

Within the control series the frequency of unequal crossing-over is low (Table 2). No heterogeneity was found either among experiments

TABLE 2. *Frequency of UCO in groups of offspring separated according to length of interval between treatment (for EDTA hatching) and oviposition.*

The significant P-values in italics.

Treatment	Days after treatment	Total nr of flies in group (n)	UCO ‰	P-value for difference from control
Control		40569	1.331	—
High temperature	< 5	26013	1.192	0.65 — 0.60
	5 — 10	41701	1.942	*0.03 — 0.02*
	> 10	17030	2.173	*0.03 — 0.02*
2500 r	< 5	7510	1.598	0.60 — 0.50
	5 — 10	19008	1.052	0.40 — 0.35
	> 10	13197	0.985	0.35 — 0.30
4000 r	< 5	10072	1.390	0.90 — 0.85
	5 — 10	36553	1.806	0.10 — 0.09
	> 10	14447	2.353	*0.02 — 0.01*
EDTA	< 10	45916	1.024	0.20 — 0.15
	> 10	17438	1.835	0.20 — 0.15

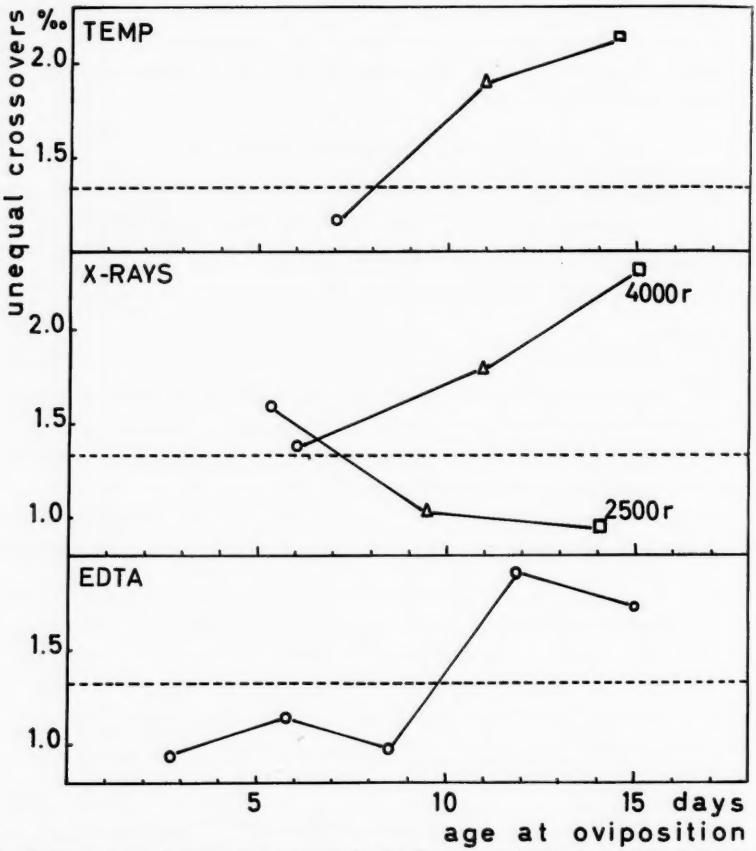

Fig. 3. Frequency of unequal crossovers in the *Bar* segment. The dashed line indicates the control frequency. Signs as in Fig. 2.

or among groups with different age of mothers at oviposition. The overall frequency of 0.1331 %, based on 40,569 scored animals, could therefore be used in comparisons with the treated series. In Fig. 3, which illustrates the effect of the treatments on frequency of UCO, the control frequency is indicated by a line.

None of the treated series showed any heterogeneity among different experiments. These have therefore been joined in Fig. 3 and in the analyses of differences shown in Table 2.

It is obvious that the temperature treatment increases the frequency of UCO in the two later intervals. Heterogeneity is present among the intervals, and in intervals 2 and 3 the frequency of UCO is significantly higher than that of the control. The X-ray treatment has had a profoundly different effect when applied in high and in low dose. The low dose, series R25, has not significantly changed the frequency from that of the control, and if anything is indicated in the results, it is a decreasing effect of the treatment. The high dose, series R40, on the other hand, has increased the frequency, and this increase is most pronounced in interval 3, i.e. in offspring from eggs laid more than 10 days after the treatment. The EDTA treatment seems to have had an increasing effect in the later broods. The two last broods taken together have a significantly higher frequency of UCO than the first three broods. However, none of them is significantly distinguished from the control.

In the general outline the frequency of UCO is affected by the treatments in the same way as the crossing-over frequency in the region *f—car*. The only exception is the last interval in the T series, where the UCO frequency is higher than the control, although the *f—car* crossing-over frequency is not increased.

3. Distribution of unequal crossover types

If unequal crossing-over is a randomly occurring meiotic event, the theoretical expectation consists of equal numbers of the four different genotypes: *f* B^+; B^+ *od car*; *f BB*; *BB od car*. However, STURTEVANT (1928) has shown that there is always an excess of round-eyed types over *double Bar*. He assumes this excess to be due to lower viability of the *BB* types and greater possibilities of their being overlooked. In the present investigation a significant discrepancy from the expected equal distribution is found if the whole material is combined (Tables 3 and 4 a). There is no heterogeneity in this respect among the treatments or among the experiments within the same series (Table 4 a); only the single experiments usually have too few cases of UCO to give significance to the discrepancy from the expected equality.

If the deficit of *BB* types is due to misclassification the results concerning the frequencies of UCO may be biased. In order to test the data, frequencies of UCO in controls and treated series were recalculated using reverted B^+ types only, but no discrepancies from the trends of the total material were found.

TABLE 3. *Distribution of UCO types and χ^2-values for discrepancies from expected equality of classes.*

Experiment No.	Distribution on UCO types				Total	χ^2
	f B+	B+ od car	f BB	BB od car		
K2T	9	4	2	5	20	5.200
K3T	4	4	3	2	13	
K2R	2	5	1	5	13	3.412
K4R	5	1	0	2	8	
Total contr.	20	14	6	14	54	7.334
T1	10	3	8	7	28	3.714
T2	5	5	3	7	20	1.600
T3	9	4	8	5	26	2.615
T4	28	21	10	16	75	9.320*
Total T	52	33	29	35	149	8.289*
R1	7	8	8	8	31	0.097
R3	11	6	7	5	29	2.862
R5	19	14	13	8	54	4.519
Total R40	37	28	28	21	114	4.526
R2	7	4	5	3	19	1.842
R4	5	7	8	6	26	0.769
Total R25	12	11	13	9	45	0.778
E1	10	10	4	11	35	3.514
E2	10	13	9	12	44	0.909
Total E	20	23	13	23	79	3.380
Total	141	109	89	102	441	13.304**

* $P<0.05$; ** $P<0.01$.

On further analyzing the total distribution it is found that the comparison between numbers of round-eyed and BB types gives the postulated excess of round-eyed types (Table 4 b). There is however also a significant excess of the two reciprocal types *f* B^+ and *BB od car* over the other reciprocal pair, B^+ *od car* and *f BB*. The pairs of reciprocal crossover types arise from the same kind of unequal pairing, viz. *f* B^+ and *BB od car*, if the left segment in the chromosome containing *f B od sy car* is paired with the right segment of the chromosome containing no mutant genes except *B*; the other pair, *f BB* and B^+ *od car*, if the right segment of the *f B od sy car* chromosome is paired with the left

TABLE 4 a. *Heterogeneity analysis of the UCO distribution from Table 3.*

	χ^2	Degrees of freedom
On sum total	13.304*	3
Heterogeneity among experiments within series	16.066	24
Heterogeneity among series	11.003	12
Total	40.373	39

* $P<0.01$.

of the *B* chromosome. Whether the observed discrepancy reflects a real difference in pairing tendency or is only to be ascribed to fortuitous viability differences cannot be judged by the present material. The viability differences clearly cannot be due to the accompanying mutant genes, for a separation according to this criterion shows no significant discrepancy.

4. Clusters

In Table 5 the numbers of UCO found in separate vials are recorded. Although there were 5 females together in each vial, the unequal crossing-over is a rare enough event for clusters to be observed if they are formed. It can be seen that three UCO is the maximum number in most experiments, and that 4-, 5-, and 6-fold events are only observed in a few vials. However, not only the number is of importance, but also as

TABLE 4 b. *χ^2-values for heterogeneity of orthogonal comparisons between pairs of types.*

Comparison	Numbers	χ^2	Degrees of freedom
Eye phenotypes; *B^+* vs. *BB*	250 — 191	7.893**	1
Pairs of reciprocal types: *f B^+* and *BB od car* vs. *f BB* and *B^+ od car*	243 — 198	4.592*	1
Accompanying mutants: *f* vs. *od car*	230 — 211	0.819	1

* $P<0.05$; ** $P<0.01$.

TABLE 5. *Number of UCO in separate vials. Comparison between actual and Poisson distribution.*

Significant P-values in italics.

Experiment No.	Distribution on vials with enumerated number of UCO							Total number of vials	Mean number of UCO per vial	Deviation from Poisson distribution		
	0	1	2	3	4	5	6			χ^2	Degrees of freedom	P-value
K2T	16	9	4	1				30	0.67			
K3T	17	11	1					29	0.45			
K2R	12	5	1	2				20	0.65			
K4R	5	3	1	1				10	0.80			
Total control	50	28	7	4				89	0.61	2.225	2	0.5—0.3
T1	16	15	5	1				37	0.76	0.408	1	0.7—0.5
T2	18	7	5	1				31	0.65	1.923	1	0.2—0.1
T3	14	8	6	2				30	0.87	1.306	1	0.3—0.2
T4	3	7	10	9	1	1	2	33	2.27	1.991	2	0.5—0.3
R1	13	13	5		2			33	0.94	0.184	2	0.95—0.90
R3	44	10	5	3				62	0.47	6.176	1	*0.02—0.01*
R5	9	11	9	7	1			37	1.46	0.469	2	0.8—0.7
R2	19	7	4		1			31	0.61	1.644	1	0.2—0.1
R4	26	13	3	1	1			44	0.59	9.255	1	0.7—0.5
E1	4	14	9	1				28	1.25	6.725	2	*0.05—0.02*
E2	11	13	6	2	2	1		35	1.26	0.61	2	0.8—0.7

to whether the multiple events comprise identical or reciprocal types or whether they are a mixture of types which cannot have a common origin.

If the occurrence of clusters of identical or reciprocal types is not directly apparent in the material, two types of statistical tests may be applied to reveal their existence. The first method is to compare the distribution of number of UCO per vial with the *Poisson* distribution. If clusters are present, vials with several UCO should be more common than what is expected according to the theoretical distribution. Since the mean number of UCO per vial differs among experiments (partly because of the enhancing influence of the treatments, partly depending on the number of broods included in the experiment), these have been tested separately. Only the control series, which is most uniform, has been combined in one test. In the χ^2-analyses the smallest classes have been added to give an expected number around 5. The result is accounted for in Table 5. Only in two of the experiments is a non-Poisson

TABLE 6. *Comparison between actual and expected distribution on identical (I), reciprocal (R), and unrelated (U) types in cases where more than one UCO appear in one vial.*

Number of coexisting UCO events	Series	Distribution on				χ^2 for diff. from expect. distribution	P-value
		I	R	U	Total		
2	Expected distr.	1	1	2	4		
	Control	3	1	3	7		
	T	4	9	13	26		
	R40	4	6	9	19		
	R25	1	2	4	7		
	E	4	3	8	15		
	Total treated	13	20	34	67	1.478	0.5 — 0.3
3	Expected distr.	1	3	12	16		
	Control	1	1	2	4		
	T	4	4	5	13		
	R40		1	9	10		
	R25			1	1		
	E		1	2	3		
	Total treated	4	6	17	27	2.086	0.2 — 0.1
4	Expected distr.	1	7	56	64		
	T			1	1		
	R40			3	3		
	R25			2	2		
	E			2	2		
	Total treated	0	0	8	8		
5	Expected distr.	1	15	240	256		
	T		1		1		
	E			1	1		
	Total treated		1	1	2		
6	Expected distr.	1	31	992	1024		
	T	1		1	2		

spread indicated, viz. in R3 and E1. From the data it is, however, apparent that the disagreement in these two cases comes from an excess of vials with only two UCO. Although such pairs may be considered as small clusters, their presence does not form any definite proof of clusters, and the distribution test must be judged as negative.

The second method implies an analysis of the coexisting types where more than one UCO is found in a vial. As has been stated before there are 4 possible types, made up of two pairs of reciprocal recombinants.

The members of each pair may have their origin from the same crossing-over event, but it must be kept in mind that following a meiotic crossing-over only one of the two reciprocal types can be recovered in the offspring, since three of the meiotic products are eliminated in the polar bodies, and only one is included in the egg cell. If, on the other hand, the crossing-over event takes place in an earlier stage and is succeeded by a number of mitotic cell divisions prior to the meiosis, both reciprocal types have chances to be included in egg cells and be transmitted to the offspring. Since the appearance of clusters is taken as an indication of such gonial crossing-over events, the *identical* (I) and the *reciprocal* (R) types are equally informative in this respect. Their frequency is to be compared to that of the cases which cannot have had a common origin, henceforth called the *unrelated* (U).

A theoretical expectation of the distribution among I, R and U can be calculated assuming independent events and thus equal probability for all four types. These expectations are given in Table 6 for the numbers from 2 to 6, which is the actual range of the co-existing unequal crossovers. In this table are also given the observed distributions in controls and the differently treated series. In the cases with 2 and 3 UCO there is no deviation from the expected distribution and thus no indication that the UCO should have a common origin and represent clusters. Of the cases with 4 UCO none could have a common origin, but one of the cases with 5 UCO is composed exclusively of two reciprocal types. The probability of this arising from independent events is 1/16. This case, as well as the two cases with 6 UCO in one vial appeared in experiment T4. One of the 6-fold appearances represents the very unlikely case of 6 identical (fB^+) individuals, which by independent origin is expected only once in 1,024 cases. The other vial with 6 UCO consisted of three from each of the reciprocal pairs, which requires at least two crossing-over events. This test thus affords some indications of clusters in this particular experiment, T4, which however did not disagree with the Poisson distribution.

Still another possibility of distinguishing between independent events and clusters is furnished by the distribution on successive broods. Since clusters are supposed to owe their origin to an induced gonial crossing-over, they cannot be supposed to appear in the offspring during the first broods. The time from the gonial stage to oviposition is estimated to be at least 6 days, and therefore UCO forming part of a cluster must appear exclusively in the later broods. This is not the case for the 6 identical $f\ B^+$ individuals from experiment T4, for these are scattered over all

broods, from the first to the last. Of the 5 reciprocal individuals from the same experiment 3 were found in the first brood, one in the second and one in the third. This consideration thus removes most of the positive evidence of the previous test. Regarding all cases where identical or reciprocal types appear together the distribution on broods is uniform and shows no excess for the later broods. Combining experiments T4, R4 and R5, where the greatest number of broods were raised, the distribution on successive broods is 19, 15, 16, 20, 16. It must therefore be inferred that no induction of clusters by the treatments used is revealed by the present data. If clusters are formed at all, they must be very rare.

IV. DISCUSSION

The enhancing effect exerted by high temperature and ionizing radiation upon crossing-over frequency is probably of the same nature, since it is effective in the same chromosomal regions, around the centromeres, and after the same characteristic time-lapse of 5 to 6 days. The effect of EDTA, on the other hand, may be of a different kind since LEVINE reports that its effect is not restricted to the proximal part of the X-chromosome. Nor is any time-limit evident, which can, however, be due to the technique of furnishing the substance. STEFFENSON and LEVINE have assumed that the increase in this case is due to a weakening of some Ca-containing chromosomal structure where exchanges of genetic material can take place.

In the case of induction with high temperature and radiation it has been inferred that the initial effect or inclination to exchanges must be brought about in the cells during the gonial stage. The nature of the effects must therefore *either* be supposed to be indirect and cause some change which remains in the cells from the time of treatment to the meiotic pachytene stage, when crossing-over has been supposed to take place, *or* it must be effective in the gonial cells and lead to a mitotic crossing-over with subsequent cell divisions and the appearance of clusters of identical or reciprocal crossovers in the offspring.

The anticipated gonial crossing-over must in essential be the same type of crossing-over as that producing mosaicism in somatic tissues, only taking place in germinal tissue and giving rise to a gonial mosaicism. Somatic crossing-over is known to occur in males as well as in females, although less frequently, and its frequency is not affected by the presence of the asynaptic *c3G* gene (LE CLERC, 1946). In contrast

to germinal crossing-over, a temperature of 30° C decreases the somatic crossing-over frequency as compared to that of 25° C (STERN and RENTSCHLER, 1936). Further, its frequency is lower than that of meiotic crossing-over by a factor of 10^4.

The low frequency and the inverted effect of high temperature make it seem improbable that the observed induction of crossing-over by temperature and radiation treatment can be solely attributed to gonial crossing-over. However, WHITTINGHILL (1955) gives conclusive evidence of gonial crossing-over events, resulting in a heterogeneous distribution of crossovers on different families and inequality in number of reciprocal classes. His material has consisted of irradiated males and females where spontaneous crossing over has been inhibited by heterozygous inversions or homozygocity for the c3G gene. Such non-random occurrence of crossovers has also been demonstrated following formaldehyde treatment of *Drosophila* males by SOBELS and STEENINS (1957). In these materials the observed crossing-over, apparently induced in the gonial stage, cannot be compared to the spontaneous meiotic crossing-over process, since this is effectively inhibited.

In other investigations of induced crossing-over there has been no intention of studying the presence of clusters, and the material has not been suited for their detection. Thus, it cannot be decided whether the observed increase is due to clusters or not. HERSKOWITZ and ABRAHAMSON (1957) have studied X-ray induced crossing-over around the centromere of the X-chromosome by means of a small duplication situated to the right of the centromere. They found a pronounced enhancing effect on crossing-over frequency, which decreased in segments farther off from the centromere, but was still appreciable in the region *f—car*. They further state that the exchanges have occurred between exactly homologous loci. The X-rays, in their opinion, do not directly exert their effect on the exchange process but the decision is brought about in cell stages earlier than the one to which spontaneous crossing-over is supposed to be limited.

In the present material both the spontaneous meiotic crossing-over and the possible formation of clusters can be studied simultaneously. No definite proofs of clusters is obtained although an increase in crossing-over is observed following treatment with high temperature, X-rays in higher doses and possibly EDTA, in the *f—car* region as well as in the much smaller *Bar* segment following unequal pairing.

The probability of obtaining clusters of unequal crossovers in the *Bar* segment can be approximately estimated as follows:

WHITTINGHILL reports that 4000 r of γ-rays gave offspring from 14 out of 59 irradiated males. Live offspring was only produced following crossing-over between *Gl* and *Sb* in the third chromosome, a region of 16 crossing-over units. Seven of the males gave from 3 to 21 offspring which could be classified as clusters. If a uniform distribution is assumed, this means a probability of 0.74 getting a gonial, cluster-producing crossing-over for each region, amounting to one unit of crossing-over in females for the given dose and for this proximal chromosome region.

Now *Bar* is about as far from the centromere as *Gl* and *Sb* and can thus be assigned to the proximal region, although rather remotely situated therein. The chance of having a spontaneous crossing-over within the *Bar* segment following unequal pairing between segments equals the average spontaneous frequency of UCO, which is 1/800. Therefore, the chance of getting a gonial crossing-over, giving rise to a cluster of unequal crossovers by a dose of 4000 r can, admittedly crudely, be estimated at $0.74 \times 1/800 = 1/1000—1/1100$ per family. Limiting the material to that treated with high temperature and the stronger X-ray dose, which were the most effective treatments, 263 vials with 5 females each give 1315 chances, and an observable cluster could reasonably be expected. No unambiguous case has however been found, which is not in itself remarkable owing to the limited material. But since the crossing-over increases are, in spite of this, obvious, the phenomenon cannot be solely attributed to cluster-producing gonial crossing-over events. There must be some other influence from the treatment which causes the observed increase in crossover frequency.

Most important in the interpretation of this conclusion is the mechanism and time of meiotic recombination. According to the commonly accepted hypothesis, originating from DARLINGTON (1935), recombination in higher organisms is effected by a breakage-reunion process after chromosome pairing in zygotene and after the time of chromosome duplication, supposed to occur in late pachytene.

New ideas concerning the process of recombination are however being put forward (for a review, see PRITCHARD, 1960), according to which the recombination is a copy-choice process taking place at the replication time of DNA, during the pre-meiotic interphase or early leptotene. Although some inconsistencies remain between this hypothesis and observed facts, the time of DNA- and chromosome-replication has been ascribed to this early stage by means of P^{32} labelling and autoradiography, both on plant materials (see TAYLOR, 1957) and on

Drosophila spermatogenesis (KAPLAN, 1960). The time has also been confirmed by studying the time of conversion from chromosome breaks to chromatid breaks (MITRA, 1958). The inconsistency between this time of recombination and the cytologically observable time of chromosome pairing has been bridged by PRITCHARD (1960). According to him the observable chromosome pairing during zygotene is not a necessary condition for recombination, which he assumes to take place in small restricted areas of effective pairing occurring at the time of chromosome replication, i.e. pre-meiotically. As evidence of this he claims the phenomenon of negative interference found in *Aspergillus* and *Neurospora.*

For the present study the hypothesis of pre-meiotic recombination is of great significance, since it brings the time of crossing-over back to the earliest oocyte or late oogonial stage, presumably the ones at which the effects of the inducing treatments are exerted. This makes superfluous the assumptions of *gonial*, mitotic, crossing-over as well as of *indirect* influence on a process taking place much later. The enhancing effect can be supposed to work directly on the recombination process during pre-meiotic interphase or earliest leptotene, 5 to 10 days before the delivery of the eggs. This probably holds both for high temperature and the effective X-ray dose. Whether the more dubious effect of EDTA is exerted in the same manner and at the same time cannot be decided as long as this treatment cannot be more exactly time-limited and quantitative.

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SUMMARY

(1) Unequal crossing-over in the *Bar* segment of *Drosophila melanogaster*, giving rise to reverted B^+ and *BB*, has been used in a study of induced crossing-over to reveal the possible presence of clusters.

(2) The following enhancing treatments have been used: high temperature, 31° C for 48 hours; X-rays in two doses, 4000 r and 2500 r; EDTA, a chelating agent known to deprive the organisms of calcium.

(3) Frequency of crossing-over in the region *f—car* has been registered and compared to the frequency of unequal crossing-over in the *Bar* segment. The two aspects of crossing-over mostly behaved in the same way in their response to the treatments. High temperature and the

higher X-ray dose were found to be effective in raising the frequency of crossovers. The lower X-ray dose was without effect and the EDTA treatment was dubious but seemed to enhance the frequency to a small extent in the later broods.

(4) The material has been tested for the presence of clusters of unequal crossovers by making comparisons with the Poisson distribution and with the expected frequencies of different kinds of crossovers assuming independent origin. Their distribution on successive broods has also been considered. The joint result of the tests was negative.

(5) Since a significant increase in crossing-over frequency has been observed without the simultaneous presence of clusters, it is inferred that induction of gonial crossing-over with subsequent multiplication of the crossover products is not the cause of the enhancing effect of the treatments.

(6) The time lapse of 5—10 days between the treatment and the observed increase traces the effective period back to the pre-leptotene stage of meiosis. It has been supposed that the influence on meiotic crossing-over is *indirect*, since the exchange has been supposed to occur in late pachytene. A recent hypothesis, however, places the crossing-over event earlier, at the time of DNA duplication in pre-leptotene, and it is suggested that, this being the case, the effect of the enhancing treatments can be *direct* on the crossing-over process that takes place at this early stage.

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KARYOTYPE ANALYSIS OF PINUS SILVESTRIS

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TWO circumstances made the present study of the chromosome morphology of *Pinus silvestris* necessary. 1) Although many cytological investigations have concerned this and other *Pinus* spp. (SAX and SAX, 1933; MERGEN and NOVOTNY, 1957; AASS, 1957; HÅKANSSON, 1956), there is no critical study of the karyotype of *Pinus silvestris*. 2) Since this species is increasingly used for radio-biological studies involving cytological observation (SUSZKA a.o., 1960), a clear understanding of its basic karyotype is essential. Whereas the lack of a satisfactory technique seems to have been the main difficulty in earlier studies, several combinations of pretreatments and fixations, applied in the present study, appeared to constitute a useful technique. As we have been able to obtain satisfactory results by our method, we hope that this description will be of value for investigators working with other gymnosperms as well.

MATERIAL AND METHODS

Primary root meristems of recently germinated seeds were used. The material was collected in Sweden from Bogesund (59°24′) and Kiruna (67°51′). Since even the metaphase chromosomes of *Pinus* are generally very long, they are easily broken with ordinary chemical pretreatments or light physical pressure during the process of squashing. Extreme care must therefore be exercised in the choice of pretreatment and pressure to be applied during the preparation of the slide. The following technique, which is a modification of the one previously used (BHADURI

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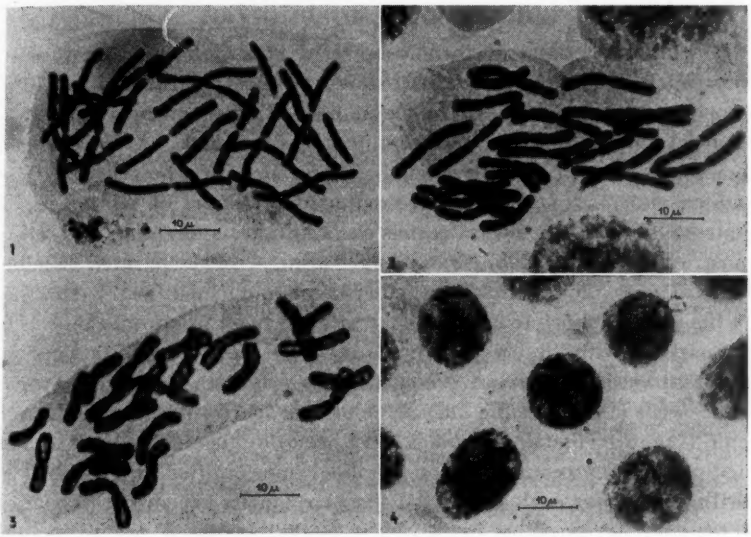
and GHOSH, 1954; BHADURI and NATARAJAN, 1956; PAI and NATARAJAN, 1958) for wheat and barley chromosomes, was applied.

1. Pretreat the root tips from recently germinated seeds with 0.2 % colchicine for four hours at 20° C.
2. Wash in water and fix in a mixture containing four parts of 2 % chromic acid, one part of 0.02 M solution of 8-hydroxyquinoline and one part of 0.2 % osmic acid (osmic acid solution being made in 2 % chromic acid) for 90 minutes.
3. Wash in water and clear in 1 % sulphuric acid for 15 minutes.
4. Wash in water and mordant in 2 % chromic acid for 60 minutes.
5. Wash in water and hydrolyse in 1 N HCl, at 60° C $\pm$ 2° C, for 45 minutes.
6. Wash and stain with leucobasic fuchsine for 30 minutes.
7. Squash the stained tip portion of the root in a drop of acetocarmine on a clean slide and make the slide permanent by freezing it over dry ice and passing it through n-butyl alcohol before mounting in euparol.

An alternative method was also adopted. It comprised pretreatment with 0.2 % colchicine solution for various lengths of time (2 to 16 hours) and fixation in Carnoy's fluid (a mixture of absolute alcohol, chloroform and acetic acid, at a ratio of 3:1:1) for six hours before changing to acetic acid—alcohol (1:3) for twelve hours. The material was brought to water through alcohol grades and hydrolysed for ten minutes in 1 N HCl, at 60° C and stained in leucobasic fuchsine, the slide being prepared and made permanent in the same way as that described above.

The main advantage of the osmic fixation is the crisp and transparent appearance of the chromosomes in which minute details of structure are revealed (Fig. 1). However, with this method the chromosomes tend to break easily, especially at the centromere region and at the region of the secondary constrictions. The chromosomes look short and swollen with Carnoy's fixation and only the main characteristics of the chromosomes could be recognized in most of the preparations. Occasionally, however, they show up well (Fig. 2). Colchicine pretreatment is extremely effective and shortens the chromosomes satisfactorily in four hours for osmium fixation (Fig. 1) and in eight hours for Carnoy's fixation (Fig. 2). A 16-hour-pretreatment brings about too much condensation of the chromosomes making the details obscure and the spiral nature of the chromosomes apparent (Fig. 3).



Figs. 1—4. Metaphase chromosomes of *Pinus silvestris.* — Fig. 1, colchicine pretreatment for 2 hours, followed by osmic fixation; Fig. 2, colchicine pretreatment for 8 hours, followed by Carnoy's fixation; Fig. 3, colchicine pretreatment for 16 hours, followed by Carnoy's fixation; Fig. 4, interphase nuclei showing a varying number of nucleoli.

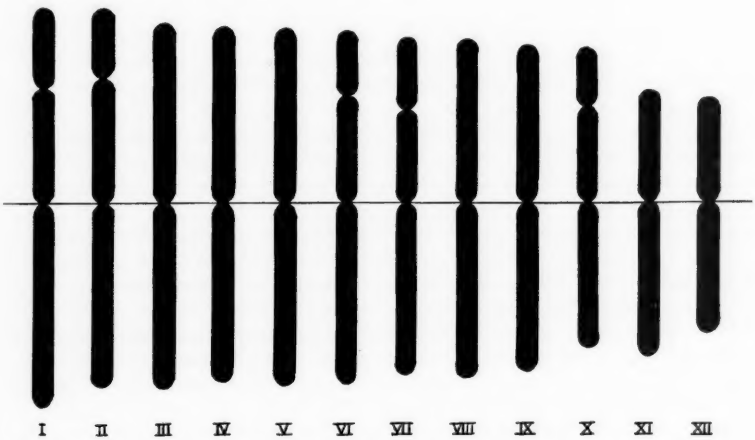


Fig. 5. Karyotype of *Pinus silvestris.*

CHROMOSOME MORPHOLOGY

All the twelve pairs of chromosomes in *Pinus silvestris* are median constricted. There are two pairs of short chromosomes without satellites (size 12—14 μ), eight pairs of medium-sized chromosomes (size 16—19 μ), of which three pairs are satellited, and two pairs of long chromosomes (size 20—21 μ) with satellites. These sizes are, however, relative and depend on the mode of pretreatment and type of fixative used. Four of the five pairs of satellited chromosomes show only secondary constrictions whereas one pair has distinct, well separated satellite heads. The two long pairs can further be distinguished from each other by the shorter short-arm and longer satellite head in chromosome I, in comparison with the longer short-arm and shorter satellite head in chromosome II. The relative measurements of five distinct metaphase plates were taken and the karyotype of this species has been drawn to scale (Fig. 5). The chromosomes have been designated as I to XII. The medium-long chromosomes, the satellited ones omitted, are difficult to distinguish from each other. All the secondarily constricted chromosomes and the satellited ones appear to be capable of organizing the nucleoli, since as many as eight to nine nucleoli have been counted in the interphase nuclei. The low number of nucleoli in most of the nuclei may be due to the fusion of several nucleoli (Fig. 4). *Pinus silvestris* is suitable for radiocytological studies, especially in view of its large-sized chromosomes. A clear understanding of the basic karyotype will therefore facilitate further analyses.

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BRIEF REPORT

A. LIMA-DE-FARIA: Classics of American Genetics.

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Anthologies in Biology are uncommon. Science is built differently from Painting or Literature. A museum is in itself an anthology of painting and, at the same time, painting itself. In literature no writer would dare to acquire his knowledge of DANTE or SHAKESPEARE from references to their writings. Contact with the original works is essential in these two fields of human endeavour.

Science is built by the permanent expansion of concepts and ideas and once the facts that an experiment produces are incorporated into the contemporary body of knowledge the original experiment becomes a piece of the whole, and does not necessarily need to be analysed in its original form.

Whereas, the fifth symphony of BEETHOVEN or the St. Anna of LEONARDO DA VINCI remain a source to which one may turn for constructive learning, one does not need to read PASTEUR's or LINNÉ's work to learn a field of biological research. The messages that the painter or the scientist deliver have an essentially similar aim — that of conveying a deeper understanding of our picture of the Universe — but the two are incorporated into it in different ways. The personality and the social background of the painter or the musician remain for ever stamped on his canvas or score, that of the scientist — although equally present — tends to fade away in the vastness of the edifice of science. For this reason an anthology in a discipline of Biology is very valuable. The reading of the original paper allows access to the personality of the scientist, his social background, as well as the development of science at that historical moment. But the value of reading the description of a classic experiment lies especially in learning the way science is built.

Professor PETER's "Classic Papers in Genetics" (Prentice-Hall, Inc. 1959) is thus a most welcome contribution. It is probably the first anthology of classic works in Genetics. Historically the writer has tried to embrace the whole span of genetic development. The first paper is MENDEL's and the last is BENZER's work.

In this series of classics, one would expect to have a representative choice made from important contributions irrespective of the scientist's nationality. Here, however, one finds a most extreme bias. Of the 28 papers included, 24 are by United States citizens. Only MENDEL, JOHANNSEN, BATESON, and HARDY are represented outside the U.S. CRICK is included in a joint paper with WATSON and PUNNETT in an article with BATESON.

If this book had been published in Nazi Germany, it would have been rightly considered a manifestation of extreme nationalism. Its publication in the U.S. does not necessarily change this judgement. That MENDEL's work was not left out of the book seems to have been due to the fact (as Professor PETERS points out) that there is an English translation. BATESON and HARDY naturally wrote in English. We must be thankful to JOHANNSEN that he wrote a summary of his paper (in German) so that the Editor could care to translate it into English.

It is always difficult to make a selection for an anthology, but this one is

highly one-sided. This is especially important since the book is primarily intended for University students who will get the conviction that genetics was "Made in America".

To argue that the papers not represented are written in languages difficult to translate would be unfair, for the Russians, the Japanese, and the Scandinavians have written in English in most cases. It is true that the French and the Germans have written in their own languages but one could at least translate their summaries, as in JOHANNSEN's case; or they could simply have been published in their original languages since the average student should be able to read them.

The names of DE VRIES, CORRENS, and TSCHERMAK are not even mentioned in the introduction or in any of the comments that precede every paper. And no mention or apology is made for the predominantly U.S. selection. Professor PETERS seems to take it for granted.

Searching through the index of "Classic papers in Genetics", one will find that not only the three papers describing the rediscovery of MENDEL's laws are missing, but also some of the most important works in the field are not included. Where are the papers by DUBININ, KARPECHENKO, NAVASHIN, TIMOFEEFF-RESSOVSKY, or VAVILOV? The papers by E. BAUR, H. BAUER, BOVERI, CORRENS, GOLDSCHMIDT, HÄMMERLING, HEITZ, OEHLKERS, RENNER, STERN, STUBBE, WEISMANN, and VON WETTSTEIN? The papers by BONNEVIE, CASPERSSON, DAHLBERG, GUSTAFSSON, HAGERUP, LEVAN, MÜNTZING, NILSSON-EHLE, or WINGE? The works of BOIVIN, CUÉNOT, EPHRUSSI, or L'HÉRITIER? The papers by AUERBACH, DARLINGTON, FISHER, GALTON, HALDANE, MATHER, PONTECORVO, or WADDINGTON? The contributions of KIHARA, MATSUURA, SINOTO, and those of BRACHET, DE VRIES, and JANSSENS? Many others could be added to this list especially since the author includes American works written as late as 1955.

One of the many examples of the oddity of the selection of papers is found in the field of Human Genetics. Most of the contributions to this field were made outside the U.S. No paper on human genetics is included; instead one finds a paper by STURTEVANT from 1954 dealing with the "Social implications of the Genetics of Man". Some papers of general interest were included but they do not represent classic experiments. Among these are STADLER's "The Gene" from 1954 and the "Genetics Conference, Committee on Atomic Casualties, National Research Council, Genetic effects of the Atomic bombs in Hiroshima and Nagasaki". Many of similar value can be found in the literature of other countries.

As an anthology of U.S. papers, the selection may be said to be well made and it is very stimulating reading. Some authors are probably over-represented (STURTEVANT, three times, and MULLER and MCCLINTOCK are each represented twice), whereas *e.g.* BLAKESLEE and BELLING are not included. Professor PETERS seems to have selected the papers partly for their didactic value and this is a valuable point of view. To anyone desiring acquaintance with the development of Genetics in the U.S., the book is strongly recommended.

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